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Effects of water deficit stress on root water relations of trembling aspen (*Populus tremuloides* Michx.) seedlings

by

Joia Aurea Siemens



A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment
of the requirements for the degree of **Master of Science**

in

Forest Biology and Management

Department of Renewable Resources

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University of Alberta

Faculty of Graduate Studies and Research

The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled **Effects of water deficit stress on root water relations of trembling aspen (*Populus tremuloides* Michx.) seedlings** submitted by **Joia Aurea Siemens** in partial fulfillment of the requirements for the degree of **Master of Science** in *Forest Biology and Management*.

ABSTRACT

The effects of water deficit on the root water relations of trembling aspen (*Populus tremuloides* Michx.) were studied in seedlings grown in sand and solution culture. Stomatal conductance, water potential, root volume flow density, and root hydraulic conductivity (L_{pr}) all declined with increasing water stress. Sand-grown aspen developed highly-branched roots and an exodermis, which may contribute to stress resistance. Tissue rehydration or changes in aquaporin activity or possibly resulted in increased root respiration.

Apoplastic flow and activation energy increased with water stress, corresponding to a decrease in aquaporin activity. Mercuric inhibition in solution culture-grown aspen was greater compared with sand-grown aspen, probably due to lack of an exodermis. Mercaptoethanol did not appear to reverse mercuric inhibition.

The pressure chamber (PC) and high-pressure flow meter (HPFM) both detected decreases in L_{pr} with increasing water stress. The HPFM is not recommended for use with water-stressed plants due to large standard errors.

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CHAPTER I

General Introduction and Literature Review

Water deficit stress is one of the major environmental factors affecting tree growth, morphology, physiology, and biochemical processes, including photosynthesis (Zahner 1968, Kozlowski 1985, Kozlowski and Pallardy 1997*b*). Water deficit stress can occur for several reasons, such as high transpiration rates under well-watered conditions, lack of water, root damage, and outplanting stresses (Kozlowski and Pallardy 1997*b*). The effects of drought stress in leaves and stems have been extensively studied in drought-stressed plants. Roots play an important role in the maintenance of tree water balance because of their role in the uptake of water and nutrients (Henzler et al. 1999). The role of roots in regulating plant water relations, particularly when subjected to environmental stresses, is not well understood (Henzler et al. 1999), because the effects of water deficit on roots have not been studied to the same extent as the effects of water deficit on leaves and stems. Additionally, much of the water deficit stress research has focused on herbaceous plants, and a greater understanding of the effects of water deficit stress on root systems of woody plants is needed.

1.1 Root Water Flow

Water travels through plants via the soil-plant-air-continuum (SPAC) (Philip 1958). Water movement through a plant is governed by a series of resistances and driving forces (Tyree and Ewers 1991). Environmental conditions, such as drought stress (Lu and Neumann 1999, Nardini and Pitt 1999), low soil temperatures (Wan et al. 1999), nutrient deficiency (Radin and Eidenbock 1984, Skinner and Radin 1994), and variable

transpirational demands can alter the rate and path of water flow (Sparks and Black 1999). Water movement from soil into roots is driven by a water potential gradient created by transpiration (Steudle and Peterson 1998). The greatest amount of resistance in this pathway is the radial movement of water through roots (Steudle and Jeschke 1983, Frensch and Steudle 1989, Melchior and Steudle 1993, North and Nobel 1996, Steudle and Peterson 1998).

1.1.1 Role of roots

Roots are an important part of SPAC since they are responsible for absorption of water lost to transpiration (Steudle and Peterson 1998). Under well-watered conditions, the rate of root water absorption is governed by their uptake efficiency which is related to: 1) total root surface area and percentage of fine roots (North and Nobel 1997, Dubrovsky et al. 1998); radial root resistance (Steudle 1994*b*, Barrowclough et al. 2000); and 3) transpirational driving forces (Dainty et al. 1981, Tyree and Ewers 1991).

1.1.2 Water movement

Movement of water into roots is driven either by osmotic or hydraulic forces (Kramer 1983). Hydraulic forces are a result of the water potential gradient created by transpiration; osmotic forces are due to the variable concentration of solutes (Steudle and Peterson 1998). In trees, hydraulic forces may be more important than osmotic forces in regulating water uptake (Steudle and Peterson 1998). When hydraulic forces are low due to reduced transpiration at night or during water deficit stress, osmotic forces may be more important for regulating water flow (Steudle and Peterson 1998). Osmotic forces

largely influence water flow across a membrane, whereas hydraulic forces can drive water flow when no membranes are present, such as through the apoplast (Steudle and Peterson 1998). The proportion of hydraulic to osmotic flow is affected by areas of variable resistance to apoplastic flow in plant tissues (Frensch et al. 1996).

1.1.3 Pathways of water movement

In roots, water is transported through the cell-to-cell and apoplastic pathways. The symplastic (through the plasmodesmata) and the transcellular (through cell membranes) pathways are classified together as the cell-to-cell pathway, since water flow through these separate pathways cannot be experimentally quantified (Steudle et al. 1993, Steudle 1994b). The apoplastic pathway (through cell walls and intercellular spaces) is the pathway of least hydraulic resistance because of high cell wall permeability when no major areas of resistance are present in roots (Henzler et al. 1999), and when hydraulic forces are greater than osmotic forces (Steudle and Peterson 1998).

In roots, most water travels through the cell-to-cell pathway because the hydraulic conductivity (L_p) of cell membranes is high due to the presence of water channels (Boyer 1985, Smith and Nobel 1986). The apoplast may be a minor pathway, based on experiments in maize (Steudle et al. 1993), barley (*Hordeum distichon*) (Steudle and Jeschke 1983), and bean (*Phaseolus coccineus*) (Steudle and Brinckmann 1989). In woody plants, suberized mature tissues may also limit apoplastic flow (Tyerman et al. 1999). However, there is considerable variability in the percentage of water travelling through the apoplastic and cell-to-cell pathways due to tissue type, plant species, and

physiological condition of the organism (Brouwer 1954, Weatherley 1982, Passioura 1988, Steudle 1994b).

The transcellular pathway, which is part of the cell-to-cell pathway, involves movement of water through cell membranes and transmembrane water channels, which will be discussed later. This pathway may be dominant when water movement through the apoplast is restricted due to areas of resistance to flow in the root tissues (Steudle and Peterson 1998).

Water can switch pathways as it travels through root systems due to high membrane permeability (Steudle 1994a, Steudle and Frensch 1996, Steudle and Peterson 1998), which may enable plants to survive and grow under changing environmental conditions (Tyerman et al. 1999).

1.1.3.1 Quantification of apoplastic flow

Numerous attempts have been made to quantify apoplastic flow with fluorescent tracer dyes (Hanson et al. 1985, Moon et al. 1986, Varney et al. 1993, Skinner and Radin 1994). It is now known that the extent of apoplastic water flow cannot be quantified with dyes confined to the apoplast because of conflicting measurements of apoplastic flow using the non-toxic apoplastic fluorescent tracer dye PTS₃ (3-hydroxy-5, 8, 10-pyrenetrisulfonate) and a cell pressure probe (Peterson et al. 1981, Hanson et al. 1985, Skinner and Radin 1994, Zimmerman and Steudle 1998). Young lateral roots in herbaceous and woody plants grow out from the pericycle and break through the exodermis and endodermis, leaving gaps that provide entry for water and PTS₃ into the root until the gaps are sealed (Dumbroff and Peirson 1971, Skinner and Radin 1994,

Steudle and Peterson 1998). Cell pressure probes can provide a measurement of cell-to-cell L_p , which can be subtracted from the value from total root L_p (L_{pr}) to obtain an estimate of apoplastic L_p (Zhu and Steudle 1991).

1.1.4 Root anatomy

Roots have a complex and variable morphological structure depending on the species, the environment, and the depth and age of the roots (Steudle and Peterson 1998). Root water flow has been compared to Ohm's Law, with water flow through plants described as a catenary process through a series of hydraulic resistors or tissues (Steudle and Peterson 1998). This analogy in plants has been experimentally validated at the tissue and cell levels (Tyree 1997). In roots, tissue types are arranged in series, from the epidermis to the xylem elements, and the extent of tissue resistance to flow determines the decrease in water potential as water crosses these areas of resistance (Steudle and Peterson 1998).

1.1.4.1 Epidermis

The epidermis is not a source of high resistance to water flow (Peterson et al. 1993), given that epidermal cells have a higher hydraulic conductivity than other cell types (Radin and Matthews 1989). Root hairs originating from epidermal cells absorb a considerable proportion of the total water absorbed by the root system (Kramer 1983).

1.1.4.2 Exodermis

The exodermis is located just below the root epidermis and may be an area of significant resistance to radial water flow (Steudle and Peterson 1998). The mature exodermis contains a Casparian band and suberin lamellae (Steudle and Peterson 1998). An exodermis is not present in roots of all plant species grown under all conditions (Zimmerman and Steudle 1998) and may not be present in young roots (Steudle and Peterson 1998).

1.1.4.3 Cortex

The cortex does not offer much resistance to water flow, based on puncturing experiments (Steudle et al. 1993). Water can move either through the apoplast, or the cell-to-cell pathways (Steudle and Peterson 1998). Similarly to the epidermis, the cortex has a high hydraulic conductivity (Radin and Matthews 1989).

1.1.4.4 Endodermis

The endodermis contains a Casparian band, and a mature endodermis consists of thickened walls and suberin lamellae on the tangential, radial, and transverse walls (Schreiber et al. 1994, Steudle and Peterson 1998, Zeier and Schreiber 1998). It is located between the cortex and the stele of the root, and acts as an area of resistance to the apoplastic pathway (Steudle and Peterson 1998). The endodermis was previously thought of as a barrier to water flow because of its hydrophobic suberin content (Kramer 1983), but it is now known that Casparian bands are comprised of hydrophilic lignin, or hydrophobic suberin deposits, or a combination of both, in the cell walls (Steudle and

Peterson 1998). Therefore, the hydrophilic materials may allow water to cross Casparian bands (Schreiber 1996, Zeier and Schreiber 1998). The endodermis is not a continuous barrier (Peterson et al. 1981) due to uneven thickenings (Kramer 1983), and immature endodermal tissue cannot effectively prevent the passage of water due to the lack of thickened walls and suberin lamellae (Steudle and Peterson 1998). Experiments measuring changes in L_{pr} following puncturing of the endodermis concluded that the endodermis is not a significant barrier to water flow (Clarkson et al. 1987, Peterson et al. 1993, Steudle et al. 1993), but there have been contradicting findings as well (Robards et al. 1973, Sanderson 1983, North and Nobel 1991, Melchior and Steudle 1993). Suberization in the endodermis, exodermis and root dermal layers could provide areas of high resistance that may be important in regulating water flow under drought stress (Cruz et al. 1992, Stasovski and Peterson 1993). However, there may also be considerable absorption of water through suberized roots given the high water and solute uptake in yellow poplar and loblolly pine (Kramer and Bullock 1966, Nobel et al. 1990, van Rees and Comerford 1990) despite the high proportion of suberized roots in mature trees (Kramer 1983).

1.1.4.5 Xylem vessels

Water crosses the vessel walls before entering the lumen and likely passes through unthickened pit membranes in the walls, which provide less flow resistance depending on their structure and composition (Steudle and Peterson 1998). Xylem vessel wall resistance is fairly low (Peterson and Steudle 1993).

In xylem elements, both tracheids and vessel members, the main driving force is hydraulic due to transpiration; resistance to axial water flow is low, particularly in mature vessels (Zhu and Steudle 1991, Steudle and Peterson 1998).

1.1.5 Composite transport model

The composite transport model describes the variation in water flow as it passes through tissues and cells of varying resistances (Steudle 1993, 1994*a, b*, Steudle and Frensch 1996, Steudle and Peterson 1998). Variability in tissue resistance may enable plants to adapt to or resist drought stress (Nardini and Pitt 1999) as a form of “coarse” or large-scale regulation (Tyerman et al. 1999) and may explain variability in root hydraulic activity (Steudle and Peterson 1998). It can also explain differences between plant species, and between herbaceous and woody plants (Tyerman et al. 1999).

In woody plants, hydraulic forces are greater than osmotic forces by three orders of magnitude, and reflection coefficients of 0.12-0.43 were found in *Quercus robur* (Steudle and Meshcheryakov 1996), and 0.22-0.82 in *Fagus sylvatica* (Steudle and Heydt 1997). In herbaceous plants, hydraulic forces are greater than osmotic forces by only an order of magnitude, and root reflection coefficients are much higher (0.35-0.98) (summarized in Steudle and Peterson 1998). Apoplastic flow, therefore, could play a larger role in woody plants, compared to herbaceous plants because of the greater hydraulic forces and low reflection coefficients (Steudle and Peterson 1998). Under conditions producing a low hydraulic gradient, it would seem that apoplastic flow in woody plants might be inefficient because of the low reflection coefficient (Steudle and Peterson 1998).

1.1.5.1 Implications for composite transport in roots

The model describes how L_{pr} can adjust in response to transpiration (Steudle and Peterson 1998), which is influenced by environmental conditions. When transpirational demand is high, the water potential gradient between soil and root xylem is high, therefore, the xylem tension in the root will be higher than the resistance to water flow through the root, and water will move radially by hydraulic force into the root system (Steudle and Peterson 1998). At night or during drought, when transpiration is low, the root resistance will be lower than xylem tension, thus preventing water movement from roots back into soil (Passioura and Tanner 1985).

1.2 Aquaporins

Aquaporins (AQPs) are a family of membrane intrinsic proteins (MIPs) that are transmembrane water channels and consist of a highly conserved amino acid sequence (Tyerman et al. 1999). Prior to their discovery, some type of water channel or membrane pore was suspected in playing a role in the high permeability of membranes since the 1960s (Dainty and Ginzburg 1964, Steudle and Tyerman 1983). Since their discovery, AQPs have been found in plant cells (Maurel et al. 1993, Daniels et al. 1994, Kammerloher et al. 1994), bacteria (Maurel et al. 1994), and animal cells (Preston et al. 1992, Yang and Verkman 1997). The first plant AQP, γ -TIP, was isolated in 1993 (Maurel et al. 1993). In plants, AQPs have been found in the plasma membranes (Daniels et al. 1994, Kammerloher et al. 1994) and in the tonoplast membranes (Maurel et al. 1993). AQPs located in the plasma membranes are called plasma membrane intrinsic proteins (PIPs), and those located in the tonoplasts are called tonoplast intrinsic

proteins (TIPs) (Johnson et al. 1990, Höfte et al. 1992, Daniels et al. 1994, Robinson et al. 1996, Chaumont et al. 1998). A considerable amount of research regarding the role of AQPs in transmembrane water transport in plants has been conducted (Chrispeels and Maurel 1994, Steudle and Henzler 1995, Maurel 1997, Schäffner 1998). To date, AQPs have been characterized in *Arabidopsis thaliana* (Maurel et al. 1993, Kammerloher et al. 1994, Daniels et al. 1996, Weig et al. 1997, Schäffner 1998), *Zea mays* (Chaumont et al. 1998), spinach (Johansson et al. 1998), *Phaseolus vulgaris* (Maurel et al. 1997a), *Mesembryanthemum crystallinum* (Yamada et al. 1995), and *Glycine max* (Weaver et al. 1994, Rivers et al. 1997). In plants, AQPs have been located in xylem parenchyma, mesophyll cells, phloem companion cells, guard cells, meristematic cells, the root cortex, and in the endodermis and pericycle (Barrieu et al. 1998a, b, Chaumont et al. 1998, Schäffner 1998).

1.2.1 Structure and function

AQPs consist of an amino acid chain forming six alpha-helices spanning a membrane, with N- and C- termini of the chain facing towards the cytoplasm (Tyerman et al. 1999). A chain has two terminal halves, each of which has a similar sequence and a hydrophobic loop that contains an asparagine-proline-alanine (NPA) section (Reizer et al. 1993, Park and Saier 1996). The NPA loops overlap within the membrane creating two “hemipores” that join to form the channel (Jung et al. 1994, Walz et al. 1995, Cheng et al. 1997). Water flow through AQPs may be bidirectional, due to channel symmetry (Cheng et al. 1997).

AQPs result in high water permeability of membranes, particularly for the tonoplasts (Maurel et al 1997b, Niemietz and Tyerman 1997). It is not known if all AQPs function as water channels in vivo (Tyerman et al. 1999) as some AQPs become inactive when isolated (Maurel et al. 1997b, Niemietz and Tyerman 1997). It is also not known if water is largely driven across channels by osmotic or hydraulic forces (Jung et al. 1994).

Some AQPs exclusively transport water across membranes (Maurel et al. 1993, 1995, Zeidel et al. 1994), while others are non-selective and will allow the passage of small neutral solutes such as urea (Echevarria et al. 1994, Ishibashi et al. 1997), and ions (Weaver et al. 1994). Therefore, not all AQPs have water transport as a primary function, particularly in more complex organisms with more than one type of AQP (Tyerman et al. 1999).

1.2.2 Permeability of AQPs to water

The direction of water flow through AQPs is determined by the ratio of osmotic permeability (P_{os}) to diffusional permeability (P_d) (Tyerman et al. 1999). Membranes that contain AQPs have a ratio much greater than one, which is the ratio for simple lipid membranes (Ye and Verkman 1989). The presence of active AQPs can be determined by measuring a membrane's osmotic water permeability, expressed as a flow rate of meters per second (Lande et al. 1995). High values for plasma membranes, $100\text{-}200 \times 10^{-6} \text{ m s}^{-1}$ (Zeidel et al. 1994) and tonoplasts, $86\text{-}600 \times 10^{-6} \text{ m s}^{-1}$ are indicative of the presence of AQPs (Maurel et al. 1997b, Niemietz and Tyerman 1997). There is no technique yet available to measure water flux through a single AQP (Tyerman et al. 1999).

1.2.3 Activation energy

This measurement of the metabolic energy requirement for water transport across cell membranes is important for understanding the role that AQP_s play in water transport. Without AQP_s, water would require energy to cross the lipid bilayer (Tyerman et al. 1999), but the activation energy (E_a) of cell membranes containing AQP_s has been found to be similar to the low E_a values of water movement by bulk flow, approximately 6 kcal mol⁻¹ (Tyerman et al. 1999). E_a has been calculated for aspen seedling roots (Wan and Zwiazek 1999). Blockage of AQP_s results in an increase of E_a (Wayne and Tazawa 1990, Shütz and Tyerman 1997). Temperature affects the membrane lipids or AQP proteins, thus interfering with the temperature-water flow relationship (Zeidel et al. 1994, Hertel and Steudle 1997).

1.2.4 Regulation of water flow

AQP_s have a system for regulating their activity and controlling water flow through the membrane, which may be similar to the open-or-closed gating mechanism for ion channels (Weaver et al. 1994, Lee et al. 1995), or may be metabolically activated or deactivated via phosphorylation (Daniels et al. 1994, Maurel et al. 1995, Johansson et al. 1998). In PM28A (Johansson et al. 1996, 1998) and a seed-specific TIP in *Phaseolus* (Johnson and Chrispeels 1992), phosphorylation is regulated in vivo by a Ca⁺²-dependent integral protein. Dephosphorylation resulted from changes in apoplastic water potential and caused reduced water permeability (Karmoker et al. 1991, Johansson et al. 1996). Regulation of water flow may occur via activation-deactivation of AQP_s, or as a result of changes in the number of AQP_s in the membrane (Tyerman et al. 1999). Inadequate

nutrient uptake of plants, including P, affects water channel activity, indicating that AQP activity is metabolically regulated (Carvajal et al. 1996). AQP regulation alone cannot account for large fluctuations in whole-plant L_p variations (Tyerman et al. 1999), therefore AQPs may act as a “fine adjustment” mechanism for controlling water flux, as opposed to the “coarse adjustment” of switching pathways (Tyerman et al. 1999).

1.2.5 Mercurial inhibition of AQPs

Some AQPs respond to blockage of the channel by mercurial reagents (Henzler and Steudle 1995, Maggio and Joly 1995, Carvajal et al. 1996, Shütz and Tyerman 1997, Maurel et al. 1997b, Niemietz and Tyerman 1997, Tazawa et al. 1996, 1997, Wan and Zwiazek 1999, Kamaluddin and Zwiazek 2001). Physical blockage occurs due to a generalized interaction between mercury and the sulphydryl groups of cysteine residues when a cysteine residue is present (Preston et al. 1992, Jung et al. 1994, Kammerloher et al. 1994, Daniels et al. 1996, Shi and Verkman 1996). Not all AQPs are mercury-sensitive because cysteine residues that are not in close proximity to the pore will not result in physical blockage of the pore upon exposure to mercurial reagents (Daniels et al. 1994). The extent of inhibition is dependent on the reagent’s ability to diffuse through the membrane and environmental factors that may affect membrane permeability (Naccache and Sha’afi 1974). $HgCl_2$, for example, can easily diffuse across a membrane because of its lipophilic nature (Tyerman et al. 1999). Blockage has been shown to reduce water flux, but not necessarily solute flux through AQPs (Chrispeels and Maurel 1994, Maurel 1997, Schäffner 1998). Cell L_p activity was inhibited by 75% or more in *Chara* (Henzler and Steudle 1995, Tazawa et al. 1996, Shütz and Tyerman 1997), 43% in

rice (*Oryza sativa*) seedlings (Lu and Neumann 1999), 47% in aspen (*Populus tremuloides*) (Wan and Zwiazek 1999), and 46-52% in dogwood (*Cornus stolonifera*) (Kamaluddin and Zwiazek 2001). Blockage may be completely reversed with the addition of sulphhydryl reducing reagents, such as 2-mercaptoethanol (ME) (Tyerman et al. 1999, Wan and Zwiazek 1999), although reversal may only be partial (Kamaluddin and Zwiazek 2001).

Mercurial reagents are not specific for a given AQP type, therefore the effects are measured are at a whole-plant or whole-root system level (Maggio and Joly 1995, Carvajal et al 1996, Tazawa et al. 1997). Mercurials, especially those which can diffuse through lipid membranes, have toxic effects (Shütz and Tyerman 1997, Steudle and Peterson 1998) and can directly interfere with plant metabolic processes such as phosphorylation (Tyerman et al. 1999). Higher concentrations of HgCl₂ have also been shown to greatly reduce root respiration in aspen (Wan and Zwiazek 1999) and dogwood (Kamaluddin and Zwiazek 2001).

1.2.6 Role of AQPs

The presence of AQPs in cells may significantly impact growth and regulatory processes of whole plants. Water is required for proper functioning of guard cells, and cell elongation and differentiation, and AQPs are necessary for efficient and rapid water diffusion across membranes (Tyerman et al. 1999). AQPs may also be important in allowing for immediate rehydration effects following long-term water stress, or for preventing xylem cavitation during drought by uncoupling xylem vessels and adjacent cells (Tyerman et al. 1999).

1.3 Diurnal Fluctuation of L_{pr}

Transpiration increases with increasing solar radiation, which causes an increase in xylem tension; transpiration, therefore, provides a hydraulic driving force for an increase in L_{pr} (Tyerman et al. 1999). Maximum L_{pr} occurred 5-7 hours after dawn in *Lotus japonicus* (Henzler et al. 1999). In the evening, with little or no solar radiation, transpiration is reduced and L_{pr} decreases; the main driving force in the absence of xylem tension is osmotic or matric (Tyerman et al. 1999). The reduced L_{pr} at night is necessary to increase root resistance to water flow and prevent water from moving back into the soil from the root system if the soil water potential is low (Passioura and Tanner 1985, Tyerman et al. 1999). Diurnal fluctuation in L_{pr} may indicate changes in the proportion of water flow through the apoplastic and cell-to-cell pathways which could involve AQPs (Steudle and Peterson 1998, Tyerman et al. 1999). Expression of mRNA for a PIP1-type AQP found in *Lotus japonicus* roots was found to be positively correlated with L_{pr} , and preceded the peak and decline of L_{pr} (Henzler et al. 1999). Cell L_p and transpiration, however, were not correlated with diurnal fluctuation in L_{pr} , therefore, AQPs may be partly responsible for changes in L_{pr} (Henzler et al. 1999).

1.4 Effects of Nutrient Deficiency on L_{pr}

Lack of nutrients affects the L_p of cells and roots. N- and P-deficient wheat roots showed an overall decrease in L_{pr} throughout the diurnal cycle (Henzler et al. 1999). In cotton roots, NO_3^- (Radin and Matthews 1989) and P (Radin and Eidenbock 1984) deficiencies caused a reduction in L_{pr} , with a decline of 80% or greater in NO_3^- -deficient plants (Radin and Matthews 1989). Barley roots also showed an 80% decrease in L_{pr}

with a SO_4^{2-} deficiency (Karmoker et al. 1991). Root size may also increase in response to nutrient stress (Henzler et al. 1999). Highly-branched and deep-rooted systems improve growth and survival in nutrient-deficient plants (Kramer 1983).

Nutrient-deficient plants can also show reductions in transpiration as a result of stomatal closure (Clarkson and Scattergood 1982, Chapin et al. 1988), however, this is not always the case when observing correlations between transpiration and L_{pr} (Carvajal et al. 1996).

1.5 Effects of Cold Temperature on L_{pr}

Decreasing temperatures result in a decline of L_{pr} (Wan et al. 2001). Less water is absorbed by roots in part because cold temperatures increase the viscosity of water (Kaufmann 1975, Wan et al. 2001) and also decrease root water permeability by affecting cell membrane properties (Henzler et al. 1999) and possibly AQP properties (Wan et al. 2001). Cold temperatures also affect plant water potential (Day et al. 1991). Wilting has been observed in orange trees that fail to absorb enough water in cold soils (Cameron 1941). This may be related to the lack of new, unsubsized root tips on plants grown in cold soils (Kramer 1983) and lack of new root growth in cold soils (Wan et al. 1999).

1.6 Effects of Drought Stress on Plants

The water balance of woody plants varies over time, depending on the season (Clark and Gibbs 1957), environmental conditions and physiological status of the plant (Tyree and Sperry 1988, North and Nobel 1996, Lo Gullo et al. 1998, Reinbott and Blevins 1999, Clarkson et al. 2000). Some of the early signs that plants are undergoing

drought stress are wilting of young leaves and stems from loss of turgor, reduced transpiration from stomatal closure, and cessation of growth (Hsiao 1973).

Water may be shuttled between different parts of the tree during drought stress. Tissues with the lowest water potentials may take water from older tissues, therefore, new leaves may experience drought stress first, but it is usually the older and lower leaves which die first because water is taken from the older tissues, and because water stress accelerates processes associated with senescence (Nooden and Leopold 1988). The lower, shaded leaves also do not produce as many carbohydrates and may be unable to adapt to prevent water losses via osmotic adjustment (Kozlowski and Pallardy 1997a).

1.6.1 Transpiration

Transpiration results in the loss of water from plants, the rate of which is influenced by the amount of solar radiation (Morison 1987, Mansfield and Atkinson 1990), stomatal aperture, and vapor pressure deficit (Grantz 1990, Schulze 1993). Stomata control the amount of water lost by plants via transpiration during drought stress (Lange et al. 1971, Schulze et al. 1972, Sena Gomes et al. 1987). Transpiration rates change in response to the water balance of a plant (Mott and Parkhurst 1991). The closure of stomata under drought stress may occur with a drop in leaf water potential due to loss of water from leaf mesophyll cells (Pierce and Raschke 1980, Schulze 1993). Stomatal closure can also be affected by ABA (Liang et al. 1997, Wan and Zwiazek 2001). The exact mechanism by which ABA affects stomata is unknown, but it may affect ion transport into and out of guard cells via transport proteins, or ion gradients across membranes (Hetherington and Quatrano 1991, Blatt and Armstrong 1993). Roots

can produce ABA under stress, and are therefore important in exposing leaves to ABA (Munns and King 1988). Roots may also influence the release of ABA from mesophyll chloroplasts (Hartung and Slovick 1991).

Some deciduous trees in mesic environments including *Populus* and *Salix* will shed some leaves during drought stress to reduce transpirational losses (Addicott 1991, Rood et al. 2000). Some plants with the majority of stomata on the underside of leaves also exhibit wilting or curling of leaves to reduce surface area to increase resistance to water flow through the leaves (Addicott 1991).

1.6.2 Diurnal fluctuation of water potential

Xylem tension increases under water deficit, which increases the resistance through SPAC, and prevents the return of leaf water potential to normal levels (Tyree and Ewers 1991). Although leaf water potential recovery may be seen in late afternoon or early evening in well-watered plants, leaf water potential in drought-stressed plants may not recover at night because of reduced soil water content and increased resistance to water flow into the root (Tyree and Ewers 1991). Under moderate drought stress, water potential at dawn is reduced; as drought progresses, plants close their stomata to prevent the drop in water potential at dawn (Bahari et al. 1985). There may be very little diurnal fluctuation in water potential in severely-stressed plants because stomata remain closed for most of the day (Pereira et al. 1986).

1.6.3 Changes in L_{pr}

L_{pr} decreases with drought stress and tends to increase with rehydration (North and Nobel 1992, 1996). L_{pr} of *Opuntia ficus-indica* (North and Nobel 1996, Dubrovsky et al. 1998) and *Sorghum bicolor* (Cruz et al. 1992) and hydraulic conductance (K_r) of *Olea oleaster* (Lo Gullo et al. 1998), decreased with soil drying. In *Oryza sativa*, root-to-leaf L_p decreased with increasing drought stress, and showed further reductions in L_p with the addition of $HgCl_2$ (Lu and Neumann 1999). L_{pr} of *Opuntia* also increased with soil rewetting but was still lower than the original L_{pr} under well-watered conditions (North and Nobel 1996). Radial conductivity (L_R) also decreased and increased with drought stress and rewatering, respectively (North and Nobel 1996). Not much is known about rehydration of mesic species since most rehydration experiments have focused on succulents and xerophytes, but the ability to recover is an important drought stress adaptation (Lo Gullo et al. 1998). It may take several days for plants to recover from severe drought stress after water becomes available (Kramer 1950, Brix 1962).

1.6.3.1 Xylem embolism

Xylem embolisms contribute to reductions in L_{pr} (Boyer 1985) and are a frequent occurrence during drought stress (Tyree and Sperry 1988, 1989, Nardini and Pitt 1999). High xylem tension develops within xylem elements, causing air to be sucked from an adjacent area into the xylem vessels through membrane pits (Crombie et al. 1985). In hardwood species, the entire water column can be disrupted (Siau 1980), and a sufficient number xylem embolisms are fatal (Tyree and Sperry 1988). The level of tension required to cause an embolism is dependent on the drought resistance of a given species

(Carlquist 1983, Tyree and Dixon 1986), and the extent of embolism depends on the rapidity of stomatal response and the transpiration rate prior to embolism (Sperry et al. 1993). Embolisms may be gradually dissolved at slight positive pressures (Tyree and Yang 1990) induced by roots or stems (Tyree and Sperry 1988) or creation of low xylem tensions (Borghetti et al. 1991, Edwards et al. 1994), thus enabling plants to recover from drought stress. However, positive pressures are not known to occur in trees (Tyree and Sperry 1988). Trees may be maximizing their photosynthetic capacity and hydraulic conductivity by operating near the threshold of catastrophic xylem dysfunction (Tyree and Sperry 1988, Tyree and Ewers 1991). Plants may be able to adapt to water stress by inducing changes in xylem transport (Tyree and Sperry 1988, Tyree and Ewers 1991).

1.6.3.2 Changes in AQP activity

AQP activity may decrease with drought stress in roots and in leaves (Lu and Neumann 1999). It is possible that AQP activity may increase slightly with moderate amounts of drought stress but decrease with severe water stress (Liu et al. 1994, Lu and Neumann 1999). Yamada et al. (1995) observed a reduction in AQP abundance in roots and leaves of *Mesembryanthemum crystallinum* with water stress. However, increases in leaf AQP expression in *Nicotiana excelsior* were observed during water and salt stress (Yamada et al. 1997). The phosphorylation and dephosphorylation of PM28A in response to changes in apoplastic water potential indicates that AQPs may play a role in the maintenance of cell turgor (Johansson et al. 1998).

1.6.4 Plant growth

Plants that undergo drought stress usually exhibit cessation or reduction in growth which can occur in shoots (Lu and Neumann 1999), and in roots (North and Nobel 1996), although shoots are more drought-sensitive (Sharp et al. 1988). Treatment of water deficit-stressed *Oryza sativa* plants with HgCl₂ caused a 49% reduction in leaf growth rates, but HgCl₂ had no effect on the leaf growth rates of well-watered rice plants (Lu and Neumann 1999). Existing root tips may also become suberized, reducing water uptake (Kramer 1983). Formation of lateral roots may increase with moderate drought stress (Cruz et al. 1992, Zhang et al. 1995, Dubrovsky et al. 1998, Lu Gullo et al. 1998, Nardini and Pitt 1999).

1.7 Drought Stress Resistance

Woody plants possess several means of drought stress resistance. There are two types of drought resistance mechanisms: drought tolerance, and drought avoidance. Woody plants are more likely to exhibit drought avoidance because of their long life span and because drought stress is usually a short-term phenomenon (Kramer and Boyer 1995). Repeated drought stress exposure causes plants to become less susceptible to drought-induced injury, therefore tree seedlings destined for outplanting are hardened by increasing sunlight exposure or pruning shoots (Allen 1955).

1.7.1 Drought tolerance mechanisms

Drought tolerance is most often seen in desert plants and some non-vascular plants and grasses, but some mechanisms may be employed by more drought-tolerant woody plants under severe or longer-term drought stress.

1.7.1.1 Cell wall elasticity

Plant cells with unligified elastic cell walls are able to contract with the loss in water volume of the cell, reducing the likelihood of cell plasmolysis (Hellkvist et al. 1974). Mature, lignified woody tissues contain rigid cell walls and are less able to contract with water loss, resulting in plasmolysis and irreversible injury (Kramer 1983). Cortical cells do shrink during drought stress (North and Nobel 1996). Giberellins may be involved in the loosening of cell wall microfibrils and synthesis of cell walls (Kozlowski and Pallardy 1997a).

1.7.1.2 Osmotic adjustment

Some cells and tissues may undergo osmotic adjustment by incorporating salts and carbohydrates into cell cytoplasm to prevent water loss from cells (Morgan 1984, Kuhns and Gjerstad 1988). Several species within the *Populus*, *Acer*, and *Quercus* genera are known to use this mechanism (Roberts et al. 1980, Parker et al. 1982, Bahari et al. 1985, Gebre and Kuhns 1991), as do some herbaceous species including rice (Lu and Neumann 1999). Drought-stressed poplars can maintain leaf turgor by altering leaf osmotic potential (Roden et al. 1990). By increasing osmotic concentrations within shoots and roots, cell water potentials decline to prevent water loss and maintain turgor

(Parker et al. 1982). Osmotic adjustment may occur in guard cells (Parker et al. 1982), chloroplasts (Berkowitz and Kroll 1988, Santakumari and Berkowitz 1991) and roots (Parker and Pallardy 1985) to preserve transpiration, photosynthesis, and plant water balance, respectively.

1.7.2 Drought avoidance mechanisms

1.7.2.1 Water storage

Woody plants postpone decreases in leaf water potential by storing water in plant tissues for short-term drought stress (Waring and Running 1978, Waring et al. 1979). Mature woody plants store some water in stems, particularly in sapwood, whereas seedlings store the majority of water in their leaves (Running 1979). Cavitation release of water from xylem can temporarily increase leaf water content, but this is a trade-off for greatly reduced L_{pr} (Tyree and Yang 1990). Seedlings also store water in both fine and main roots to prevent leaf desiccation (Pallardy et al. 1982).

1.7.2.2 Transpirational control

Woody plants exert some control over transpiration during drought stress. Leaf abscission and branch die-back may occur during severe stress to prevent additional water loss at the expense of photosynthesis (Parker and Pallardy 1985, Rood et al. 2000). Plants may develop smaller or more dissected leaves (Hinckley et al. 1981), or thicker cuticular wax (Pallardy and Kozlowski 1980). Longer-term stress is also associated with increased cuticular thickness in comparing poplars grown in the field with poplars grown

in growth chambers (Pallardy and Kozlowski 1980). Closure of stomata occurs due to decreases in leaf water potential, thereby decreasing transpiration and acting as a first defense mechanism against xylem dysfunction (Tyree and Sperry 1988, Sperry et al. 1993). The sensitivity of stomatal closure in response to drought stress is determined by drought resistance of tree species, age of leaves, and exposure to previous drought stress (Davies and Kozlowski 1977, Abrams 1990, Ni and Pallardy 1991, Kubiske and Abrams 1992)

1.7.2.3 Tissue resistance

Increased resistance to water flow through tissues also increases drought stress resistance (Lo Gullo et al. 1998). Tissue resistance is greater in some drought-tolerant species and was correlated with low transpiration rates and resistance to xylem cavitation in Eastern red cedar (Ginter-Whitehouse et al. 1983). However, low internal water potential gradients between roots and leaves seem to be used to maintain high water flux for a given transpirational level, thereby preventing large decreases in water potential to avoid water stress (Tyree et al. 1991). Increased resistance to root water flow may be related to the increased suberization of tissues in the exodermis and endodermis following drought stress which may occur closer to the root apex (Cruz et al. 1992, North and Nobel 1996, Lo Gullo et al. 1998); this could slow the recovery of L_{pr} following rewatering (Lo Gullo et al. 1998). Increased resistance to water permeability in roots has also been associated with slight losses in cell turgor (Kramer 1983). Resistance can prevent tissues from undergoing rapid reductions in water potential (Tyree et al. 1991).

1.7.2.4 Root systems

Woody plants with deeper root systems, greater branching, and more fine roots are better able to deal with drought by increasing root-soil contact and absorbing more water from soil (Hinckley et al. 1981, Stone and Kalisz 1991, Nardini and Pitt 1999). Root-soil gaps created during soil drying contribute to the increased resistance to water flow and reduced uptake during drought stress (Huck et al. 1970, Faiz and Weatherley 1982, Taylor and Willatt 1983, North and Nobel 1997). Root system size also greatly increases in *Arabidopsis thaliana* when AQP expression is greatly reduced (Kaldenhoff et al. 1998). In *Vicia faba*, the majority of root water uptake occurred from 1.5 to 8 cm behind the root tip (Kramer 1983) therefore a greater number of root tips increases water uptake. More root growth occurs in moderately drought-stressed plants than shoot growth, which could increase water uptake (Sharp and Davies 1979, Keyes and Grier 1981, Gower et al. 1992, Dubrovsky et al. 1998). The length of time over which root growth continues and the drought-induced mortality of root tips is greater with gradual soil drying, but lateral root formation is more important during rapid soil drying (Dubrovsky et al. 1998). Recovery from drought stress following rewatering has been associated with the resumed growth of root tips and the formation of new lateral roots and root hairs (North and Nobel 1996, Dubrovsky et al. 1998, Lo Gullo et al. 1998), because young root tips have greater L_{pr} than older root sections (Frensch and Steudle 1989, North and Nobel 1992).

The response of L_{pr} to drought stress and to seasonal changes reflects species' drought adaptation strategies. Drought-tolerant tree species may reduce L_{pr} during water stress, whereas drought-avoiding species may increase L_{pr} to absorb more water from the

soil and compensate for increased transpirational losses during hot and dry weather (Nardini and Pitt 1999).

1.7.2.5 Xylem vessels

Xylem has greater conducting ability than is required for water transport, which allows for a certain percentage of xylem vessels to be embolized due to drought without greatly affecting water transport (Tyree and Sperry 1988). Decreases in water potential in drought-resistant or avoidant species encourage and increase water uptake to maintain high water content, resulting in a lower number of embolisms when water is available (Nardini and Pitt 1999). For drought-tolerant species, which may encounter low water availability, it is a better strategy to be less vulnerable to xylem cavitation than to have high xylem efficiency (Nardini and Pitt 1999). For example, narrower vessels are more cavitation-resistant (Salleo and Lo Gullo 1986, Sperry and Saliendra 1994). Water also continues to move through unblocked vessels in the event of embolisms (Scholander et al. 1957) although the resulting tension in the unblocked vessels is increased (Tyree and Sperry 1988). Trees may have means of isolating vital organs from embolisms in more peripheral, expendable organs (Zimmerman 1983, Rood et al. 2000) by confining cavitation (Tyree and Sperry 1988, Sperry et al. 1993, Nardini and Pitt 1999)

1.8 Study Objectives

The review of literature has identified many gaps in our understanding of the effects of drought and drought resistance mechanisms in woody plants. The role of roots in drought resistance mechanisms has received little attention. Roots are important in

water uptake and transport in plants and the recent discovery of aquaporins and subsequent studies have pointed to the possibility that these structures may participate in regulation of root water flow. Therefore, the present study has focused on the properties of root water flow and the function of aquaporins in trembling aspen (*Populus tremuloides* Michx.) seedlings subjected to water deficit stress. Trembling aspen were used for this study because they are a commercially valuable species and have been used in land reclamation efforts. Aspen can grow sizeable root systems in a short period of time, which makes them suitable for use in multiple experiments.

The principal objectives of this present study were to:

- (1) Examine the effects of water deficit stress on the properties of root water flow in trembling aspen (*Populus tremuloides*) seedlings.
- (2) Examine the effects of water deficit stress on root aquaporin activity.
- (3) Compare the pressure-chamber method and high-pressure flow meter method used for the measurement of root hydraulic conductivity (L_{pr}).
- (4) Compare the root morphology and the effects of water deficit stress on the water relations parameters of seedlings grown in sand and solution culture.

I studied the hypotheses that:

- (1) Increasing levels of water deficit stress would cause a reduction in seedling root water flow.
- (2) Water deficit stress would decrease activity of root AQPs.
- (3) Aspen seedlings grown in sand and solution culture would have root anatomical and morphological differences. Sand-grown aspen roots are

more likely to possess drought-resistant morphology than solution culture-grown aspen roots.

- (4) The pressure chamber would produce more consistent measurements of L_{pr} than the high-pressure flow meter (HPFM).

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Chapter II

Effects of Water Deficit Stress and Recovery on the Root Water Relations of Trembling Aspen (*Populus tremuloides*) Seedlings

2.1 INTRODUCTION

Water deficits in sand- and soil-grown plants can occur due to numerous factors including lack of water, seedling outplanting stress due to root damage and lack of root-soil contact, and high transpirational demand. Resulting water deficit stress limits growth and physiological function of woody plants (Kramer 1983, Sperry et al. 1993, Henzler et al. 1999). Roots play a major role in the regulation of water uptake, and therefore, in the maintenance of plant water balance (Steudle and Peterson 1998, Kozlowski and Pallardy 1997). However, the regulation of root water absorption, particularly in woody plants and under conditions of water stress, is not well understood (Henzler et al. 1999).

Radial movement of water through roots occurs via the apoplastic (outside of protoplasts) pathway and the cell-to-cell pathway; the latter is comprised of the symplastic (through plasmodesmata) and the transcellular (through cell membranes) pathways (Steudle and Peterson 1988). Although the cell-to-cell pathway may be dominant in many plants (Boyer 1985, Smith and Nobel 1986, Tyerman et al. 1999), the proportion of water flux through these pathways may change with drought and other stresses (Steudle 1994, Steudle and Frensch 1996). The dominant pathway is partly dependent on the presence of areas of high resistance to water flux, such as the endodermis (Schreiber et al. 1994, Schreiber 1996, Zeier and Schreiber 1998) and exodermis (Steudle and Peterson 1998), but there has been conflicting evidence as to the

role of the exodermis and endodermis in regulating water flow (North and Nobel 1991, Peterson et al. 1993, Steudle et al. 1993, Melchior and Steudle 1993). Aquaporins (AQPs) in plant roots play a role in the transcellular pathway (Chrispeels and Maurel 1994, Maurel et al. 1997, Niemietz and Tyerman 1997), and could also play a role in water uptake regulation during drought stress. Some AQPs appear to be regulated by phosphorylation (Daniels et al. 1994, Maurel et al. 1995, Johansson et al. 1998). It is not known how drought stress affects AQPs, although AQP deactivation might occur with a drop in apoplastic water potential (Karmoker et al. 1991). Because AQPs allow for high water permeability of cell membranes, AQP deactivation could increase the activation energy required for water to move through cell membranes, and result in an increase in apoplastic root water flow.

Apoplastic fluorescent tracer dyes such as PTS₃ (3-hydroxy-5, 8, 10-pyrenetrisulfonate) have been used to quantify apoplastic flow (Moon et al. 1986, Skinner and Radin 1994, Maggio and Joly 1995), with varying success. Although dyes may not accurately measure apoplastic flow (Peterson et al. 1981, Hanson et al. 1985, Zimmerman and Steudle 1998), they may still provide useful information about the proportion of water flux through the apoplastic pathway under drought stress. Mercurial inhibition of AQPs (Henzler and Steudle 1995, Maggio and Joly 1995, Carvajal et al. 1996, Wan and Zwiazek 1999, Kamaluddin and Zwiazek 2001) could be used to measure AQP activity during drought stress.

The objective of this study was to examine the effects of water deficit stress on the root water relations of trembling aspen seedlings and on the activity of root AQPs. We tested the hypothesis that an increasing water deficit would cause a decrease in root

hydraulic conductivity. We also hypothesized that AQP activity would be lower in water-stressed seedlings to conserve intracellular water and this would, in turn, result in an increase in the proportion of apoplastic root water flow, and an increase in activation energy.

2.2 MATERIALS AND METHODS

2.2.1 *Effect of water deficit stress on root water relations*

2.2.1.1 *Plant culture*

Trembling aspen (*Populus tremuloides* Michx.) seedlings were grown from seed collected from ten open-pollinated clones in Whitecourt, Alberta, Canada. Seeds were germinated in Petri dishes for one week in distilled water. Germinants were planted into foam polymer-filled styroblock trays (Oasis potting medium, Beaver Plastics, Edmonton, AB) and grown for one month in a controlled environment growth chamber under the following conditions: temperature, 20°C (day)/ 20°C (night); relative humidity (RH), 45%; and 16-h photoperiod with photosynthetically active radiation of 265 $\mu\text{mol s}^{-1} \text{m}^{-2}$ at plant height. Seedlings were bottom-watered daily and bottom-fertilized once a week with 0.3% 20-20-20 (N-P-K) fertilizer solution. Two-month-old seedlings were removed from the trays and the roots were cleaned of foam medium. Seedlings were planted two per pot into 3-L plastic pots lined with landscaping fabric to allow for drainage, and filled with non-sterile washed sand. Sand was obtained from the Faculty of Agriculture, Forestry, and Home Economics at the University of Alberta. Coarse sand was used as a

potting material to allow for rapid induction of water deficit stress. Seedlings were grown for two months in the controlled environment growth chamber, with the same growth conditions and same watering schedule as above. Pots were randomly rotated once a week within the growth chamber to minimize the effects of light intensity gradients within the chamber.

2.2.1.2 Water deficit stress treatments

Three levels of water deficit stress were applied to the four-month-old seedlings: mild stress (MS), severe stress (SS), and severe stress recovery (SR). The MS and SS levels were used to determine if aspen response would differ between the two levels. The SR treatment was used to determine if aspen could recover from the SS treatment. A control group (CTRL) consisted of seedlings that were watered daily with tap water. Mild water deficit stress was achieved by withholding water until seedlings showed mild signs of leaf wilting. Severe water deficit stress was induced by withholding water until severe wilting of leaves was observed. At 45% RH, it took three days to achieve mild stress, and four days to achieve severe stress. Severe stress recovery was achieved by watering SS seedlings 24 h before measurements were taken. A minimum of six plants was used for each stress and control group to provide a sufficient sample size for statistical analysis.

2.2.1.3 Stomatal conductance and water potential

Stomatal conductance (g_s) was measured for intact plants within the growth chamber using a LI-1600 steady-state porometer (LI-COR, Lincoln, NE) as described by

Wan and Zwiazek (1999). For each plant, one fully-expanded, uninjured leaf from approximately the middle of the stem, was selected for measurements.

Water potential (ψ_w) was measured in excised shoots using a Scholander pressure chamber, as described by Wan et al. (1999).

2.2.1.4 Root hydraulic conductivity

In the first experiment, hydraulic conductivity was measured using a Scholander pressure chamber (PMS instruments, Corvallis, OR) as previously described (Henzler et al. 1999, Wan and Zwiazek 1999). In this experiment, six seedlings were used for each stress treatment group, and for the control group. Measurements of g_s and ψ_w were taken for each plant to quantify the level of water deficit stress imposed as described previously, but measurements were not shown in this study. Detopped root systems were immersed in distilled water at room temperature inside the pressure chamber, with the cut section outside of the pressure chamber. The chamber was initially pressurized with compressed air at 0.25 MPa, and root flow rate was allowed to equilibrate for five minutes prior to measurements. Steady-state root flow rates were quantified by attaching a graduated glass pipette to each of the cut sections of the root systems with a short section of rubber tubing to measure volume flow changes over time. After measuring steady-state root flow rate for 20 minutes, pressure was increased to 0.95 MPa, at 0.05 MPa increments. At each pressure, root flow rate was allowed to stabilize and steady-state flow rate was measured for 20 minutes.

To account for differences in water flow due to differences in root system size, root systems were washed and surface-dried, wrapped in aluminum foil, frozen in liquid

nitrogen and broken into smaller fragments. The total surface area of the roots was measured following computerized scanning (Sigma Scan 4.0, Jandel Scientific, San Rafael, CA). Root flow rates at each pressure were expressed as volume flow density, J_v ($\text{m}^3 \text{ m}^{-2} \text{ s}^{-1}$). Root hydraulic conductivity (L_{pr}), expressed as $\text{m}^3 \text{ m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$, was obtained from the regression line of J_v plotted against hydrostatic pressure.

The root hydraulic conductivity experiment was repeated three times, but the results from only one of the experiments are shown in this study.

2.2.1.5 Root respiration

Root respiration rates of control and stressed seedlings were measured as the rate of O_2 consumption over time, using a dissolved oxygen meter with a Clark-type electrode (YSI 5000, Yellow Springs Instruments, Yellow Springs, OH). Root systems were placed in an airtight plexiglass container filled with distilled water, with the electrode immersed at half-depth in the water. The container was placed into a shallow basin of water to minimize temperature effects due to the magnetic stirrer, and the water within the sealed container was magnetically stirred for the duration of the measurements. After a two-minute equilibration time, dissolved O_2 concentrations were measured at two-minute intervals for the first ten minutes, then at five minute-intervals for the next ten minutes. Root respiration rate was calculated as the difference in O_2 concentration over time and expressed on a root area basis ($\text{mg O}_2 \text{ L}^{-1} \text{ m}^{-2} \text{ s}^{-1}$).

Root respiration measurements were repeated once in a separate experiment, but only the respiration results obtained for plants which were used in the root hydraulic conductivity experiments are shown in this study.

2.2.1.6 Sand water content

Samples of sand were taken from all pots at the time seedlings were measured and harvested and weighed to obtain wet weight. Samples were oven-dried at 86°C for 24h and re-weighed to obtain oven-dried weight. Sand water content for all treatments was calculated from wet and dry weights of samples.

2.2.2 Effects of HgCl₂ and mercaptoethanol on root water relations

In the second experiment, aspen seedlings were grown from seed collected from Edmonton, Alberta. The seedlings were grown under the conditions described above, although the RH in the growth chamber was approximately 60%. Water deficit stress treatments lasted for 14 days for MS and 18 days for SS treatments. Sample sizes for the control, MS, SS, and SR groups were 8, 7, 7, and 6 seedlings, respectively. The increased time required to induce water deficit stress in this study was due to an increase in the RH of the growth chambers. As in the first experiment, SR treatment was achieved by rewatering seedlings for 24 h. Measurements of ψ_w and g_s were taken for this experiment as described earlier. Measurements of ψ_w , g_s , and initial J_v values were used to illustrate the relationships of these three parameters shown in this study.

To detect changes in the proportion of apoplastic water transport, 0.02% (w/v) solution of 3-hydroxy-5, 8, 10-pyrenetrisulfonate (PTS₃) was used in place of distilled water for root water flow rate measurements. PTS₃ is a nonionic fluorescent dye used as a tracer of apoplastic transport (Hanson et al. 1985, Moon et al. 1986, Skinner and Radin 1994).

2.2.2.1 Root volume flow density

Roots were immersed in the Scholander pressure chamber in a 0.02% PTS₃ solution and root volume flow density (J_v) was quantified as described for the root hydraulic conductivity experiment. In this experiment, root flow rates were allowed to equilibrate for 20 minutes prior to initial root flow measurements. Root flow was measured using 1.0 MPa of pressure over a period of 60 minutes. Xylem sap from pressurized roots was collected for dye concentration analysis.

Following measurement of initial water flow rates, roots were removed from the pressure chambers, the solution was aerated, and sufficient HgCl₂ was added to give a final concentration of 0.1 mM HgCl₂ (Wan and Zwiazek 1999). HgCl₂ is known to reduce water channel activity by reducing movement of cell-to-cell water flow through the transmembrane water channels (Maggio and Joly 1995, Maurel et al. 1997). The medium was stirred with a magnetic stirrer, and roots were placed back into the medium and into the pressure chamber. Pressure was gradually increased to 1.0 MPa, and held for 20 minutes before root flow measurements were taken. Root flow rate after the addition of HgCl₂ was measured for 60 minutes and xylem exudates were collected for the analysis of PTS₃ concentration.

Following water flow measurements in HgCl₂-treated roots, the roots were removed from the pressure chamber and the solution was aerated before adding 2-mercaptoethanol (ME) to the HgCl₂ and PTS₃ solution to give a final concentration of 50 mM ME. Pressure was gradually increased to 1.0 MPa and root systems were pressurized for 20 minutes prior to water flow rate measurements. Xylem exudates were collected for the analysis of PTS₃ concentration after 60 minutes. For all stressed and

control seedlings, initial J_v values, and J_v values after the additions of HgCl_2 , and of ME were calculated as described for the root hydraulic conductivity experiment.

This experiment involving use of HgCl_2 was repeated three times, and repeated once with the subsequent use of ME. These repeated experiments used a lower pressure of 0.3 MPa, but the results of these repeated experiments are not shown in this study. A pressure of 0.3 MPa has been used previously in the literature (Wan and Zwiazek 1999, Kamaluddin and Zwiazek 2001), but in the present study, 1.0 MPa was used because HgCl_2 was observed to have a significant effect on AQP activity at this pressure.

2.2.2.2 PTS₃ concentration analysis

PTS₃ concentrations in xylem exudate samples were measured using a Sequoia-Turner 450 fluorometer (Apple Scientific, Chesterland, OH) with a 405 nm excitation and 515 nm emission (Skinner and Radin 1994). A standard curve was constructed using known concentrations of PTS₃. Samples were diluted in distilled water to measure PTS₃ concentrations, and actual xylem exudate concentrations were calculated using the dilution factors. Apoplastic flow was estimated by dividing xylem sap PTS₃ concentrations by the concentration of PTS₃ used in the root solution medium (Kamaluddin and Zwiazek 2001).

Measurements of PTS₃ concentration were also conducted for two repeated experiments involving the use of HgCl_2 on root systems at lower pressures of 0.3 MPa. The results of these repeated experiments are not shown in this study.

2.2.3 Activation energy

In a third experiment, the effects of water deficit stress on the activation energy required for root water transport were measured. Water deficit stress treatments were induced as described above. Sample sizes for the control, MS, SS, and SR treatments were 7, 7, 8, and 8 seedlings, respectively. At 60% RH, it took 15 and 19 days to induce MS and SS stress, respectively. ψ_w and g_s measurements were conducted as described above, but were not shown in this study.

A high-pressure flow meter (HPFM, Dynamax Inc., Houston, TX) was used to measure hydraulic conductance of root systems at different temperatures (Tyree et al. 1995). Roots systems were immersed in a 500-mL beaker of distilled water, which was continuously aerated. The beaker was placed in a circulating water bath (Frigomix B, B. Braun, Melsungen, Germany). A digital thermometer was used to measure the temperature of the water within the beaker. Hydraulic conductance measurements were taken at 20, 16, 13, 10, 7, and 4°C, in descending order, then again in ascending order. With each change in temperature, roots were exposed to the new temperature for approximately five minutes prior to taking hydraulic conductance measurements. Hydraulic conductance values were expressed over root surface area to calculate root hydraulic conductivity, $L_{pr(H)}$ ($\text{kg m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$). These values were used to produce an Arrhenius plot for each seedling by plotting the natural logarithm of $L_{pr(H)}$ against the reciprocal of temperature. Activation energy, (E_a , kcal mol^{-1}) was calculated using the equation: $E_a = - [R \times (\ln K_2 - \ln K_1) / (T_2^{-1} - T_1^{-1})]$

where $R = 1.987 \times 10^{-3} \text{ kcal mol}^{-1} \text{ T}^{-1}$

$K_2 = L_{pr}$ at time 2

$K_1 = L_{pr}$ at time 1

T_2 = temperature (°K) at time 2

T_1 = temperature (°K) at time 1

2.2.4 Statistical analysis

For the root hydraulic conductivity and activation energy experiments, differences in the mean L_{pr} , respiration, and E_a values between the treatment and control groups were tested for statistical significance using SPSS 10.0 (SPSS Inc. Chicago, IL) to perform univariate ANOVA. For the $HgCl_2$ and PTS_3 experiment, univariate ANOVA was also used to test for statistically significant differences between the mean initial J_v values and between mean initial percent apoplastic flow values of treatment and control groups. For the $HgCl_2$ and PTS_3 experiment, paired-t tests were used to test the differences between initial J_v , J_v after the addition of $HgCl_2$, and J_v after the addition of ME within each treatment or control group. Paired-t tests were also similarly used to test for differences in PTS_3 concentration and in percent apoplastic flow between initial values and values after the additions of $HgCl_2$ and ME within each treatment or control group. Linear regression analyses ($y = mx + b$) were conducted to analyze the significance of the relationships between g_s , ψ_w and J_v , which were part of the root hydraulic conductivity experiment. Paired-t tests and linear regressions were conducted using SPSS. For all statistical tests, the critical p-value was set at 0.05.

The statistical model used for univariate ANOVA using a completely randomized design was: $Y_{ij} = \mu + t_i + e_{ij}$

where Y_{ij} = value of individual observation (i = treatment, j = observation)

μ = overall mean

t_i = effect of i^{th} treatment (where i = level of water deficit stress)

e_{ij} = random error

2.3 RESULTS

2.3.1 Stomatal conductance, shoot water potential and root volume flow density

Stomatal conductance (g_s), shoot water potential (ψ_w), and initial root volume flow density (J_v) measurements shown in Figs. 2.1A-C are from the experiment described in section 2.2.2. Stomatal conductance (g_s) in aspen seedlings decreased significantly ($p \leq 0.05$) with decreasing shoot water potential (ψ_w) (Fig. 2.1A). Similarly, root volume flow density (J_v) significantly ($p \leq 0.05$) decreased with decreasing ψ_w (Fig. 2.1B) and with decreasing g_s (Fig. 2.1C). In three repeated experiments, g_s did not significantly decrease in mildly-stressed (MS) seedlings and showed little recovery in stress-recovered (SR) seedlings compared with severely-stressed (SS) seedlings (data not shown). Similarly, little effect of MS treatment was observed on ψ_w , but in three experiments, the ψ_w of SR seedlings returned to the control level (approximately -0.5 MPa) within 24 h following rewatering. ψ_w of SS seedlings in all experiments reached between -2.0 MPa to -4.0 MPa (data not shown).

2.3.2 Root hydraulic conductivity

The mean slopes of the relationship between J_v and pressure from 0.25 MPa to 0.95 MPa were used to calculate root hydraulic conductivity (L_{pr}) (Fig. 2.2A). L_{pr} values

were similar in control and MS seedlings (Fig. 2.2B). In severely-stressed plants (SS), L_{pr} was approximately one-third that of control plants (Fig. 2.2B). SR plants did not show significant recovery after 24 h of rewatering (Fig. 2.2B).

Measurements of g_s and ψ_w from this experiment were not shown.

2.3.3 Effects of HgCl₂ and mercaptoethanol on root water relations

Results of J_v measurements are shown as normalized values (Fig. 2.3A) to adjust for differences in initial J_v values, which are the J_v measurements taken prior to addition of HgCl₂ to the incubation solution. Because initial J_v values are normalized, means of initial J_v do not have standard error bars. As in three repeated experiments, which were not shown in this study, initial J_v values of SS and SR treatments decreased significantly ($p \leq 0.05$) compared to control seedlings (data not shown). There were no significant differences between initial J_v values of SS and SR seedlings, and between MS and control seedlings in this study. Initial J_v values of the control, MS, SS, and SR treatments were 12.3, 13.2, 2.9 and 2.9 $\text{m}^3 \text{m}^{-2} \text{s}^{-1} \times 10^{-8}$, respectively. Following the addition of HgCl₂, normalized J_v in control and MS seedlings was reduced by 20.7% and 15.5% respectively, although the latter difference was not significant (Fig. 2.3A). Decreases in normalized J_v were observed in SS and SR seedlings, with reductions of 19.1% and 29.0%, respectively (Fig. 2.3A). Only the decreases in control and SR seedlings due to the addition of HgCl₂ were significant ($p \leq 0.05$). Mercaptoethanol (50 mM ME) added to the HgCl₂ solution significantly ($p \leq 0.05$) reduced normalized J_v in control and MS seedlings by 42.7% and 40.8%, respectively, compared to normalized J_v of roots treated with HgCl₂ (Fig. 2.3A). ME also further reduced normalized J_v of SS and SR seedlings

by 8.0% and 29.5%, respectively, but only the latter decrease was significant ($p \leq 0.05$) (Fig. 2.3A). In repeated experiments where J_v was measured at 0.3 MPa, there was no significant consistent effect of HgCl_2 and ME on J_v values (data not shown).

Measurements of g_s and ψ_w also obtained from this experiment are shown in Figs. 2.1B and C.

2.3.4 PTS_3 concentration in xylem exudate

PTS_3 concentrations are shown in Fig. 2.3B as normalized values to adjust for differences in initial values, therefore initial PTS_3 concentration means do not have standard error bars. Initial PTS_3 concentrations measured prior to the addition of HgCl_2 increased in the SS treatment relative to control seedlings, but due to large variation in the measurements, only the initial value for the SS treatment was barely significant ($p=0.054$) compared to the MS treatment (data not shown). This increase in SS seedlings relative to controls was also observed in two repeated experiments (data not shown). MS and SR treatments had PTS_3 concentrations similar to controls (data not shown). Initial PTS_3 concentrations in the control, MS, SS, and SR treatments were 2.6, 1.9, 4.0, and $2.6\% \times 10^{-3}$, respectively. Normalized PTS_3 concentrations increased in control and MS seedlings in response to the addition of HgCl_2 (Fig. 2.3B), and in response to the addition of ME to the HgCl_2 solution (Fig. 2.3B), but differences were not significant. The addition of HgCl_2 to SS and SR seedlings resulted in decreases in normalized PTS_3 concentration of 30.3% and 19.4%, respectively, but only the former was barely significant ($p=0.054$). In response to the addition of ME to the HgCl_2 solution,

normalized PTS_3 concentrations in SS and SR seedlings increased, but were not significantly different from, the PTS_3 concentrations following addition of HgCl_2 .

PTS_3 concentrations significantly ($p \leq 0.05$) increased with decreasing ψ_w (Fig. 2.4A) and with increasing J_v (Fig. 2.4B).

Apoplastic flow values were calculated from PTS_3 concentrations as a percentage of total root flow. Apoplastic flow increased in SS seedlings compared to MS seedlings, but the difference was barely significant ($p = 0.054$) (Table 2.1). All other differences in percent apoplastic flow due to the addition of HgCl_2 between treatment and control groups were not significant (Table 2.1). There were also no significant increases in apoplastic flow due to the addition of ME for any treatment or control groups (Table 2.1). In a repeat experiment using 0.3 MPa of pressure, PTS_3 concentration and percent apoplastic flow did not significantly change following the addition of HgCl_2 for all stress and control treatments (data not shown).

2.3.5 Activation energy

Root hydraulic conductivity (L_{pr}) showed a linear relationship with changing temperature (Figure 2.5). Descending Arrhenius plots of MS and SS treatments had a greater slope compared with C and SR treatments (Fig. 2.5). This resulted in larger mean activation energy (E_a) values compared with C and SR plants (Fig. 2.6). However, due to large standard errors, the differences in the mean E_a values between the different treatments and control groups were not significant.

2.3.6 Root respiration

Respiration significantly ($p \leq 0.05$) increased in MS and SS seedlings compared to control seedlings, and SR seedlings returned to control root respiration values following 24 h of rewatering (Fig. 2.7).

The relationships between root respiration and L_{pr} (Fig. 2.8A) and between respiration and ψ_w (Fig. 2.8B) were weak and therefore were not significant.

2.4 DISCUSSION

2.4.1 Effect of water deficit stress on root water relations

In the present study, severe water deficit stress resulted in a reduction in g_s and shoot ψ_w , which was correlated with a decline in L_{pr} (Fig. 2.1). There was some recovery of g_s and ψ_w in seedlings following rewatering for 24 h, although full recovery was not observed in this study (data not shown). The observed reduction in g_s may be necessary for aspen seedlings to prevent excessive water loss from seedlings by reducing stomatal aperture, and to prevent xylem cavitation (Kramer 1983, Sperry et al. 1993, Kozlowski and Pallardy 1997). It has been previously observed that shoots are more drought-sensitive than roots (Sharp et al. 1988). The positive correlations between g_s , ψ_w , and L_{pr} (Fig. 2.1) may indicate that stomatal closure could have been triggered by a decrease in leaf and shoot water potential (Pierce and Raschke 1980, Schulze 1993), or by the presence of abscisic acid (ABA). ABA can be produced in the roots and does not affect root water flow, but has been shown to induce stomatal closure (Liang et al. 1997, Wan and Zwiazek 2001).

In other studies, it has been observed that drought stress reduces stomatal conductance (g_s) and water potential (ψ_w) of plants (Mott and Parkhurst 1991, Liang et al. 1997). In *Populus trichocarpa*, the time required to induce severe drought stress was dependent upon the populations' drought resistance (Sparks and Black 1999). Recovery has been observed in stressed plants that have been rewatered (North and Nobel 1992, 1996, Lo Gullo et al. 1998), although recovery may take several days depending on the severity of water deficit (Kramer 1950, Brix 1962, North and Nobel 1996). In comparison with studies involving woody plants, values of g_s , ψ_w , and J_v in control seedlings in this study were similar to those obtained in other experiments involving control groups of trembling aspen and dogwood (*Cornus stolonifera*) seedlings subjected to similar growth conditions (Wan et al. 1999, Wan and Zwiazek 1999, 2001, Kamaluddin and Zwiazek 2001).

From this study, it was found that aspen might be able to tolerate moderate amounts of water deficit stress. Mild water deficit stress did not have a significant effect on ψ_w or g_s (data not shown) or L_{pr} (Fig. 2.2B) as these values were not significant from those of the control. In a few repeated experiments which were not shown in this study, non-significant increases in g_s and L_{pr} were observed in mildly-stressed seedlings compared to controls, even though ψ_w in those seedlings was consistently lower than controls in all experiments. These findings may indicate that ψ_w is a more accurate indicator of water deficit stress than g_s or L_{pr} . In this study, mild stress seemed to produce more variability than severe stress in the response of plant water relations to water deficit stress, indicated by the larger standard errors for g_s , J_v , L_{pr} , and E_a within a given experiment, even when sample sizes for different treatments were the same. Slight

increases in g_s could occur in poplars via osmotic adjustment to maintain turgor in the guard cells (Parker et al. 1982, Kuhns and Gjerstad 1988, Roden et al. 1990) which would increase stomatal aperture, and therefore increase g_s . This increase could explain the observed decline in ψ_w . The transpirational gradient is one of the main driving forces for water uptake, (Steudle and Peterson 1998, Henzler et al. 1999), and it is thought that the rate of stomatal conductance controls the rate of root water flux, according to the cohesion-tension theory (Steudle 2001). A slight increase in g_s could have resulted in a slight increase in L_{pr} , which could have allowed for increased water absorption from drying soil. Since woody plant seedlings can store additional water in their roots (Pallardy et al. 1982), and root cells can also undergo osmotic adjustment, an increase in g_s and L_{pr} could have been temporarily sustained even with a lack of water. Additionally, it was observed that although induction of mild stress required several days, the time required to induce severe stress was only slightly longer than that required for mild stress, supporting the fact that aspen may be able to tolerate slight water deficit stress. The results indicate that aspen may have a variable response to water deficit depending on the level of stress imposed.

The results from the present study show that root water flow, both J_v (Figs 2.1B, C) and L_{pr} (Fig. 2.2B), were reduced as a result of increasing water deficit stress. This was observed in several repeated experiments (data not shown). The reduction in root water flow was likely as a result of increased xylem embolism, or possibly due to increased tissue resistance in stressed seedlings. As xylem tension increases due to continuation of g_s and a lack of water to sustain g_s , xylem embolisms could have formed, further reducing root water flow through xylem. Xylem embolisms result in reduction of

water flow, stomatal closure, and wilting (Boyer 1985, Nardini and Pitt 1999, Tyree and Sperry 1988, 1989). Although pressure was used in the present study to induce flow rates, it is likely that embolisms were still present in xylem and were not all flushed from the xylem, as the xylem exudate was full of air bubbles. Diurnal fluctuation studies have shown that L_{pr} decreases at night to prevent water flux from roots back into soil under reduced transpirational flow (Passioura and Tanner 1985, Tyerman et al. 1999). During water deficit stress when g_s , and therefore hydraulic forces are low, L_{pr} may also decrease to prevent water loss from roots via osmotic forces (Tyerman et al. 1999). Therefore, increased tissue resistance in response to water deficit stress could have reduced water flow through roots. Areas of high resistance in the roots, such as cell wall thickenings and increased suberization of root tips and the endodermis, can develop with gradual drought stress (North and Nobel 1996, Lo Gullo et al. 1998, Cruz et al. 1992).

Several experiments, most of which used non-woody plants, have previously shown that root water flow measurements, including hydraulic conductance (K_r) and hydraulic conductivity (L_p) have decreased in response to water deficit stress (Cruz et al. 1992, North and Nobel 1992, 1996, Dubrovsky et al. 1998, Lo Gullo et al. 1998, Lu and Neumann 1999, Martre et al. 2001), and have increased following rehydration (North and Nobel 1992, 1996, Martre et al. 2001). Because most of these water deficit stress studies used non-woody plants, the results of this study cannot be directly compared to non-woody plant studies with respect to time required for induction of water deficit stress or rehydration and L_{pr} values.

Slight recovery of L_{pr} (Fig. 2.2.2B) and ψ_w (not shown) was observed in the present study, indicating that aspen can recover from the level of severe stress induced in

this experiment. No other previous experiments involving water deficit stress on woody plants have examined stress recovery. Recovery of L_{pr} in aspen could have been explained by new lateral root formation and removal of embolisms. Lateral roots, which are formed during rapid soil drying, could also contribute to stress recovery when water becomes available (Keyes and Grier 1981, Gower et al. 1992, North and Nobel 1996, Dubrovsky et al. 1998, Lo Gullo et al. 1998). In this study, root microscopy of control and stressed seedling roots were not compared, therefore it is not possible to determine if lateral root formation played a role in stress recovery. There is also some evidence that plants may be able to induce positive pressure from the roots in order to dissolve embolisms, although to date there is lack of evidence for this process in woody plants (Tyree and Sperry 1988).

2.4.2 Effects of $HgCl_2$ and mercaptoethanol on root water relations

In the present study, significant mercuric inhibition of J_v was observed in control (20.7%) and stress-recovered (29.0%) sand-grown aspen seedlings, but there was no significant inhibition in mildly- or severely-stressed seedlings (Fig. 2.3A). $HgCl_2$ is known to reduce L_{pr} by physically blocking the flow of water through AQPs (Preston et al. 1992, Chrispeels and Maurel 1994, Jung et al. 1994, Kammerloher et al. 1994, Maurel 1997, Schäffner 1998). During observation of diurnal fluctuation of L_{pr} it was discovered that genetic AQP expression in *Lotus japonicus* corresponded with and preceded increases in L_{pr} (Henzler et al. 1999) therefore the effect of AQP activity on measurements of root water flow might be significant.

Several experiments have demonstrated mercuric inhibition of water flow in plants (Henzler and Steudle 1995, Maggio and Joly 1995, Carvajal et al. 1996, Shütz and Tyerman 1997, Maurel et al. 1997), although most experiments involving the study of AQPs have used non-woody plants. In solution culture-grown aspen and dogwood, reductions in root water flux (measured at 0.3 MPa) due to HgCl_2 were 47% for J_v (Wan and Zwiazek 1999) and 46-52% for steady-state root flow rate (Q_v , $\text{m}^3 \text{s}^{-1}$) (Kamaluddin and Zwiazek 2001), respectively. Therefore, the results from this study confirm that the AQPs within root systems of trembling aspen are mercury-sensitive, indicated by significant inhibition in control seedlings.

The lack of mercuric inhibition in severely-stressed seedlings could likely be the result of reduced metabolic activity in root systems as a result of water deficit stress. When AQPs are active, as in control seedlings, they contribute to high membrane water permeability (Maurel et al. 1997, Niemietz and Tyerman 1997). Some AQPs are metabolically regulated via enzymatic phosphorylation (Johnson and Chrispeels 1992, Daniels et al. 1994, Maurel et al. 1995, Johansson et al. 1996, 1998), meaning that phosphorylation renders an AQP active, and dephosphorylation renders an AQP inactive. Zhang and Tyerman (1999) have shown that HgCl_2 did not have an effect on wheat root cells that were less metabolically active. AQP deactivation has been shown to occur in response to changes in apoplastic water potential (Karmoker et al. 1991) and reduced mercuric inhibition has been observed in severely water deficit-stressed plants (Martre et al. 2001). Therefore, severe water deficit stress may have resulted in some deactivation of AQPs (Fig. 2.3B) and contributed to a reduction in L_{pr} (Fig. 2.2B) in the present study. AQPs may become deactivated in response to water deficit stress, when hydraulic forces

are low, to prevent excess water loss from cells and from root systems via osmotic forces. If AQPs were largely deactivated as a result of water deficit stress, then addition of HgCl_2 to deactivated AQPs would not likely result in further inhibition of water flow through AQPs. The fact that stress-recovered seedlings showed significant mercuric inhibition similar to controls indicates that AQPs can be regulated, and may help aspen to recover from water deficit stress by becoming active.

The role of root anatomy may influence the results of mercuric inhibition experiments. In previous experiments which used solution culture-grown aspen and dogwood, respectively (Wan and Zwiazek 1999, Kamaluddin and Zwiazek 2001), significant mercuric inhibition was attained with the low HgCl_2 concentrations used in this study at pressures of 0.3 MPa. In the present study, no significant inhibition was observed when the experiment was repeated at pressures of 0.3 MPa (data not shown). However, mercuric inhibition was obtained in sand-grown aspen seedlings at 1.0 MPa. Root microscopy has revealed that aspen grown in solution culture possess an endodermis, but lack an exodermis (Wan and Zwiazek 2001). There is evidence that sand-grown aspen roots possess an exodermis (Chapter III in this thesis), which could be an adaptation of sand-grown aspen to growing in sand. For plants grown in solution culture, water is not a limiting growth factor. Because the growth medium is fully hydrated, solution culture-grown aspen may not need adaptations such as an exodermis to prevent excess water loss from roots. Although the function of the exodermis is unclear, this study suggests that it may provide an area of resistance to flow of water and possibly solutes. The presence of the exodermis in sand-grown aspen in this study may also explain why percent mercuric inhibition was lower in this study, compared with percent

inhibition obtained with solution culture-grown woody plants (Wan and Zwiazek 1999, Kamaluddin and Zwiazek 2001). Although HgCl_2 can diffuse through cell membranes (Shütz and Tyerman 1997, Steudle and Peterson 1998), greater hydrostatic pressures may be required for HgCl_2 to permeate sand-grown aspen roots because of the presence of the exodermis. Water deficit-stressed plant roots can also produce cell wall thickenings and additional suberization in the endodermis and exodermis (Cruz et al. 1992, North and Nobel 1996, Lo Gullo et al. 1998), resulting in added resistance to root water flow through stressed root systems and potentially resulting in reduced mercuric inhibition of AQPs.

Mercaptoethanol (ME), which has been used to reverse mercuric inhibition (Tyerman et al. 1999, Wan and Zwiazek 1999), failed to reverse mercuric inhibition in all stress treatments and controls in the present study (Figs. 2.3A, B) when similar concentrations (50 mM) were used as those used in the literature. Although ME has previously restored J_v to 91% of original values prior to the addition of HgCl_2 , ME has not always been able to restore measurements of root water flux (Zhang and Tyerman 1999, Kamaluddin and Zwiazek 2001). ME itself is toxic and an inhibitor of metabolic activity. Its toxic effects are indicated by the significant decrease in J_v following application of ME to control and mildly-stressed seedlings (Fig. 2.3A). After this study was conducted, it was observed in an experiment conducted in this lab that 5-20 mM of ME is an optimal concentration for inducing reversal of mercuric inhibition, whereas 50 mM greatly reduced metabolic activity (Kamaluddin, unpublished results). Therefore, concentrations of ME used in this study were likely too high to observe reversal of mercuric inhibition.

2.4.3 PTS_3 concentration in xylem exudate

Previous studies have shown that apoplastic flow, as a percent of total root flow quantified by apoplastic tracer dyes, was 2% or less (Hanson et al. 1985, Moon et al. 1986, Skinner and Radin 1994, Wan and Zwiazek 1999, Kamaluddin and Zwiazek 2001), indicating that most of the water flux occurred through the symplast, perhaps due to the presence of the Casparian band (Skinner and Radin 1994). With the addition of the metabolic inhibitors $HgCl_2$ and NaN_3 , percent apoplastic flow increased from 0.01% to 0.025%, and from 1 to 4%, respectively, in aspen (Wan and Zwiazek 1999) and dogwood (Kamaluddin and Zwiazek 2001) seedlings.

In the present study, PTS_3 concentration increased with the addition of $HgCl_2$, and of ME, although these differences were not sufficiently large to be definitely significant (Fig. 2.3B). Because apoplastic flow estimates are directly related to PTS_3 concentration, the results indicate that apoplastic flow increased with the addition of $HgCl_2$ and of ME, and these increases corresponded with decreases in J_v following the addition of $HgCl_2$ and of ME in control and mildly-stressed seedlings (Fig. 2.3A). Because the reduction in J_v was related to AQP deactivation (Fig. 2.3A), the results suggest that apoplastic flow increases with the blockage of AQPs by $HgCl_2$. Some metabolic inhibition of root water flow with the addition of ME seems to have resulted in an increase in apoplastic flow as well. Because PTS_3 concentration was higher in severely-stressed and stress-recovered seedlings than in controls (data not shown), the results suggest that apoplastic flow increased with increasing water deficit stress as a result of AQP deactivation in water deficit-stressed seedlings. The fact that apoplastic flow increased with water deficit

stress, indicated by the significant decline in the water relations parameters ψ_w and J_v , is also shown in Figs. 2.4A and B.

Apoplastic flow rates in the present study were approximately 12.8% for controls and 9.3% for mildly-stressed plants (Table 2.1), which were considerably higher than the 1% apoplastic flow observed in previous experiments. Previous experiments, however, used seedlings grown in solution culture (Skinner and Radin 1994, Wan and Zwiazek 1999, Kamaluddin and Zwiazek 2001), or partially-flooded conditions (Moon et al. 1986). The presence of the exodermis, and additional suberized tissues, which are present in sand-grown aspen, but not necessarily present in sand-grown plants, could potentially result in a lower percent apoplastic flow by forcing water to move through cell membranes. The removal of sand-grown aspen roots from sand could have disturbed or damaged the roots, allowing water to enter the root systems through fissures in the exodermis or suberized tissues. It has been observed that root systems that are disturbed by removing them from pots of sand and placing them into solution, exhibit larger percentages of apoplastic flow, from 13% in mangrove (*Avicennia marina*) (Moon et al. 1986) up to 52% in cotton (*Gossypium hirsutum*) (Skinner and Radin 1994). Steudle and Peterson (1998) theorized that the increases in PTS_3 dye uptake in disturbed plants could occur due to the breakthrough of lateral roots where the endodermis was not yet mature. Therefore seedlings grown in solid potting medium may be more susceptible to disturbance or damage than seedlings in solution culture.

It should be noted that percentages of apoplastic flow calculated from PTS_3 concentrations in xylem exudate are estimates of the actual apoplastic flow rates, not actual values. Tracer dye molecules are much larger than water molecules, and therefore

dye movement through root systems would be different from that of water (Hanson et al. 1985). PTS₃ cannot move through AQPs, as the pore diameter is only slightly larger than the diameter of water (Tyerman et al. 1999). The larger diameter of PTS₃ may also render it unable to move through some of the apoplastic spaces through which water can move. The Casparian band may not be a significant barrier to water, but may still prove to be a substantial barrier to solute molecules such as PTS₃. Therefore, the concentration of PTS₃ that is able to enter the xylem exudate through the apoplast may underestimate the actual percent apoplastic flow of water.

2.4.4 Activation energy

AQPs allow for high water permeability with low energy expenditures required for water to move through cell membranes containing AQPs. Low E_a values ($<6 \text{ kcal mol}^{-1}$) indicate the presence of transmembrane AQPs (Tyerman et al. 1999). In the present study, E_a was high in water deficit-stressed seedlings, and was low in control and stress-recovered seedlings (Fig. 2.6), indicating that AQPs were indeed deactivated in stressed seedlings. Higher E_a values indicate energy expenditures associated with unmediated water movement across membranes, and with AQP deactivation (Wayne and Tazawa 1990, Shütz and Tyerman 1997). The fact that means were not significantly different between treatment and control groups may be related to the use of the HPFM. Usage of the HPFM for measuring root hydraulic conductivity ($L_{pr(H)}$) has resulted in high standard errors, although it can detect changes in $L_{pr(H)}$ between stress treatments and controls (Chapter IV in this study). Lack of significance may be due to small sample size, or possibly due to repeated measures of $L_{pr(H)}$, which could result in damage of

tissues or inaccuracies in subsequent measurements on the same root system (problems with the HPFM are discussed in Chapters IV and V). Therefore, the greater resistance and higher energy expenditures required for water to flow through cell membranes suggests greater AQP deactivation in stressed seedlings. This is consistent with the evidence of AQP deactivation in stressed seedlings from the mercuric inhibition and apoplastic flow experiments.

2.4.5 Root respiration

Root respiration has been theoretically linked to AQP activity (Wan et al. 2001), due to the metabolic regulation of some AQPs via phosphorylation. Respiration rates in previous studies have decreased with the addition of HgCl_2 and NaN_3 (Wan and Zwiazek 1999, Kamaluddin and Zwiazek 2001), which suggest a relationship between metabolic inhibition or AQP deactivation and respiration rates. In the present study, root respiration increased with water deficit stress (Fig. 2.7), while L_{pr} and AQP activity decreased with water deficit stress (Figs. 2.3A, B). Although root respiration increased with increasing water deficit stress, the relationship between respiration and ψ_w or J_v was too variable to be significant (Figs. 2.8 A, B). The fact that respiration increased in stressed seedlings may be the result of changes in AQP activity. Phosphorylation and dephosphorylation of AQPs may be metabolically controlled and therefore would require energy expenditures. AQP activity can also be controlled by genetic expression of AQPs (Henzler et al. 1999), which determines the number of AQPs present. It is possible that the increased respiration in stressed seedlings reflects a change in AQP activity, but it is not possible to determine if this was the case in the present study based on the results alone. Further

experiments would be necessary to confirm the relationship between respiration rate and AQP expression or activity.

Another possibility for increased respiration is that some localized tissue rehydration occurred during respiration measurements. Root systems were immersed in distilled water, with a dissolved oxygen probe used to measure the decline in dissolved O₂ concentration. Water-stressed aspen root cells, which may have accumulated metabolic solutes via osmotic adjustment (Kramer 1983, Morgan 1984, Kunns and Gjerstad 1988), may have become more metabolically active with the presence of water than control seedlings, thus increasing root respiration rates. Because L_{pr} measurements take into account the whole-root response to water deficit stress, L_{pr} could have remained low in water deficit-stressed seedlings in the short term, while L_p of water-stressed root cells could have increased with an increase in metabolic activity.

In conclusion, the results of this study showed that water deficit stress resulted in a reduction of g_s , ψ_w , and L_{pr} . AQPs present in aspen roots are mercury-sensitive, indicated by the significant reduction in J_v in control seedlings following the addition of HgCl₂. Water deficit stress seemed to result in AQP deactivation, indicated by lack of mercuric inhibition, and in an increase in percent apoplastic flow in aspen roots. The results suggest that AQPs may play a substantial role in regulation of water flow through cell membranes, and that AQPs do show some ability to regulate root water flow during water deficit stress. AQP deactivation may be a mechanism used to prevent excess water loss from root systems by reducing water flow through cell membranes. AQP deactivation during water deficit stress also results in an increased E_a , indicating that

there is increased resistance to flow across membranes. It was found that sand-grown aspen requires higher hydrostatic pressures to induce mercuric inhibition, which is likely due to the presence of an exodermis. Increased respiration rates in water deficit-stressed seedlings may be due to changes in AQP activity or AQP expression, but further experimentation is required to understand the relationship between AQP activity and root respiration rate. Because most water deficit stress and AQP activity experiments have focused on non-woody plant species, this study is important in that it provides information regarding the effects of different levels of water deficit stress and recovery, and regarding AQP activity relating to water deficit stress for a commercially-important woody plant species.

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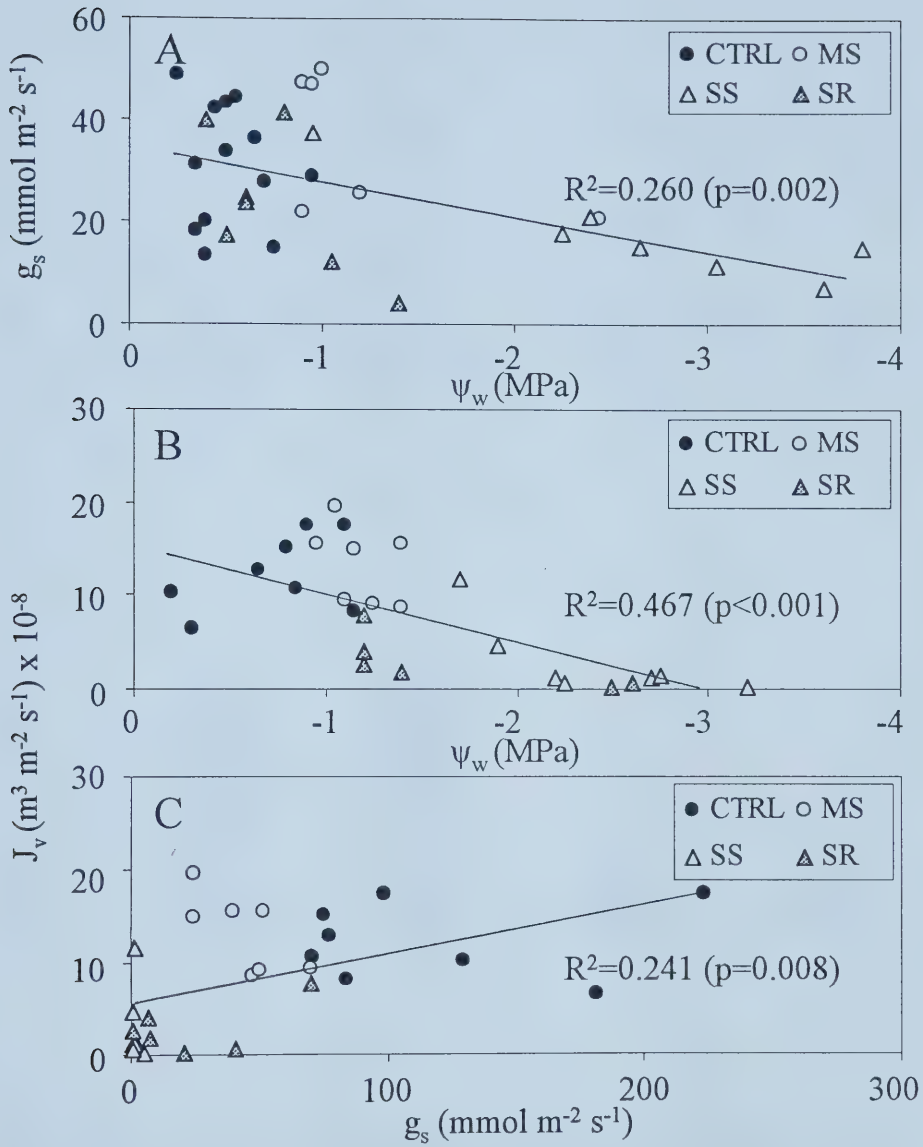


Figure 2.1. Relationships between water potential (ψ_w) and stomatal conductance (g_s) (A), between ψ_w and root volume flow density (J_v) (B), and between g_s and J_v (C) in aspen seedlings. Control (CTRL), mildly-stressed (MS), severely-stressed (SS), and stress-recovered (SR) treatments are shown. All regressions are significant at $p \leq 0.05$.

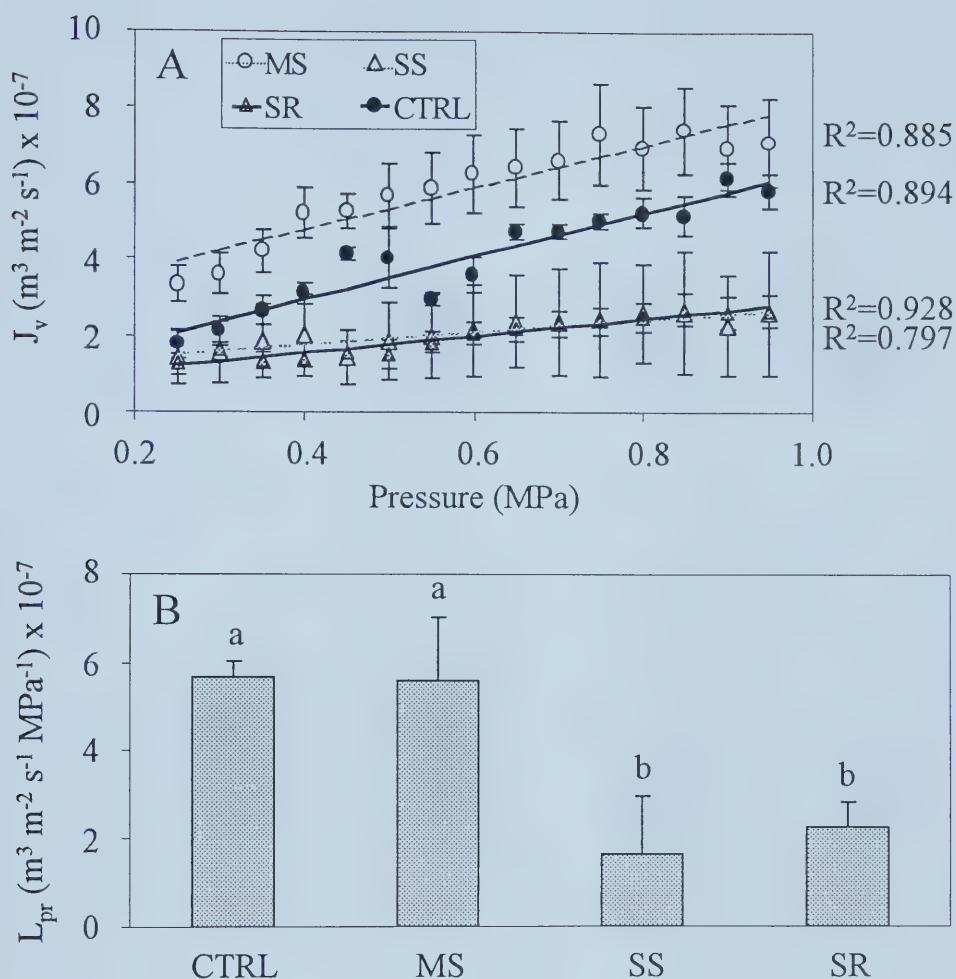


Figure 2.2. (A) Relationship between J_v and hydrostatic pressure for control, mildly-stressed, severely-stressed, and stress-recovered aspen seedlings. Means \pm SE are shown. Linear regressions are significant (all $p < 0.001$). (B) Root hydraulic conductivity (L_{pr}) of control (CTRL), mildly-stressed (MS), severely-stressed (SS), and stress-recovered (SR) aspen seedlings. Means \pm SE are shown (all $n=6$). Different letters indicate significant differences.

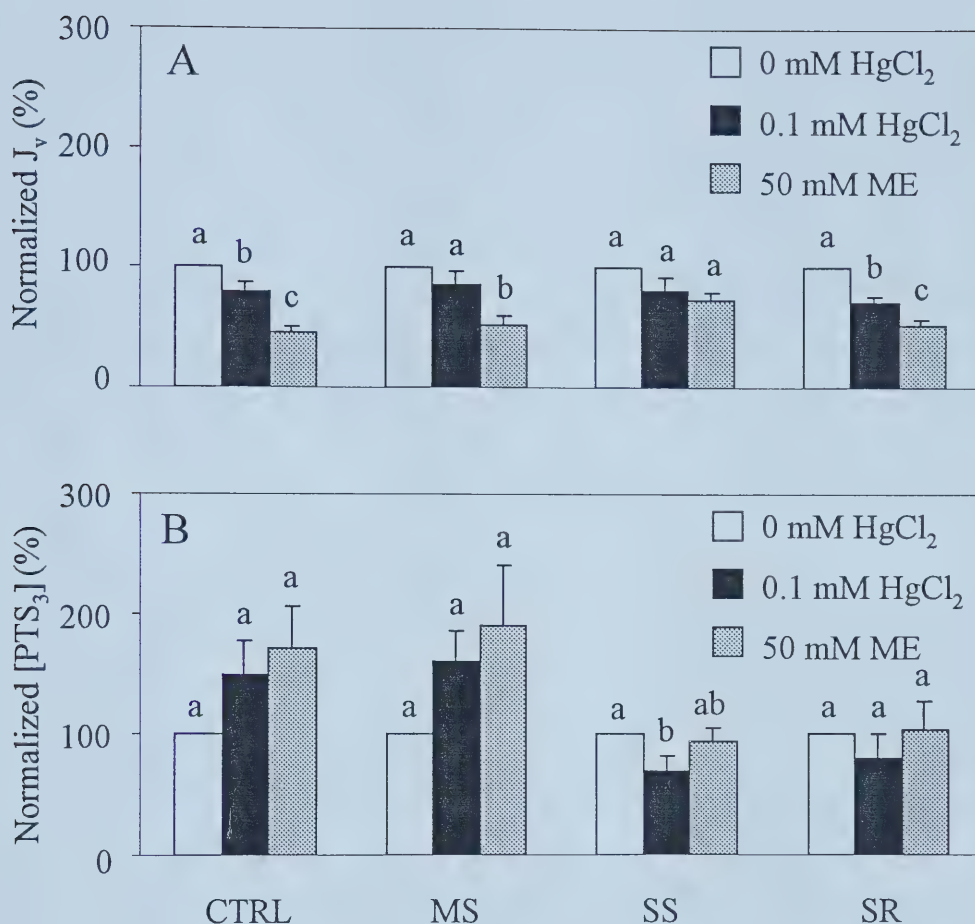


Figure 2.3. Effect of HgCl_2 and 2-mercaptoethanol (ME) on root volume flow density (J_v) (A) and PTS_3 concentration in xylem exudate (B) of control (CTRL), mildly-stressed (MS), severely-stressed (SS), and stress-recovered (SR) aspen grown in sand. Bars with different letters within each treatment are significantly different ($p \leq 0.05$). Means + SE are shown ($n_{\text{CTRL}}=8$, $n_{\text{MS}}=7$, $n_{\text{SS}}=7$, $n_{\text{SR}}=6$).

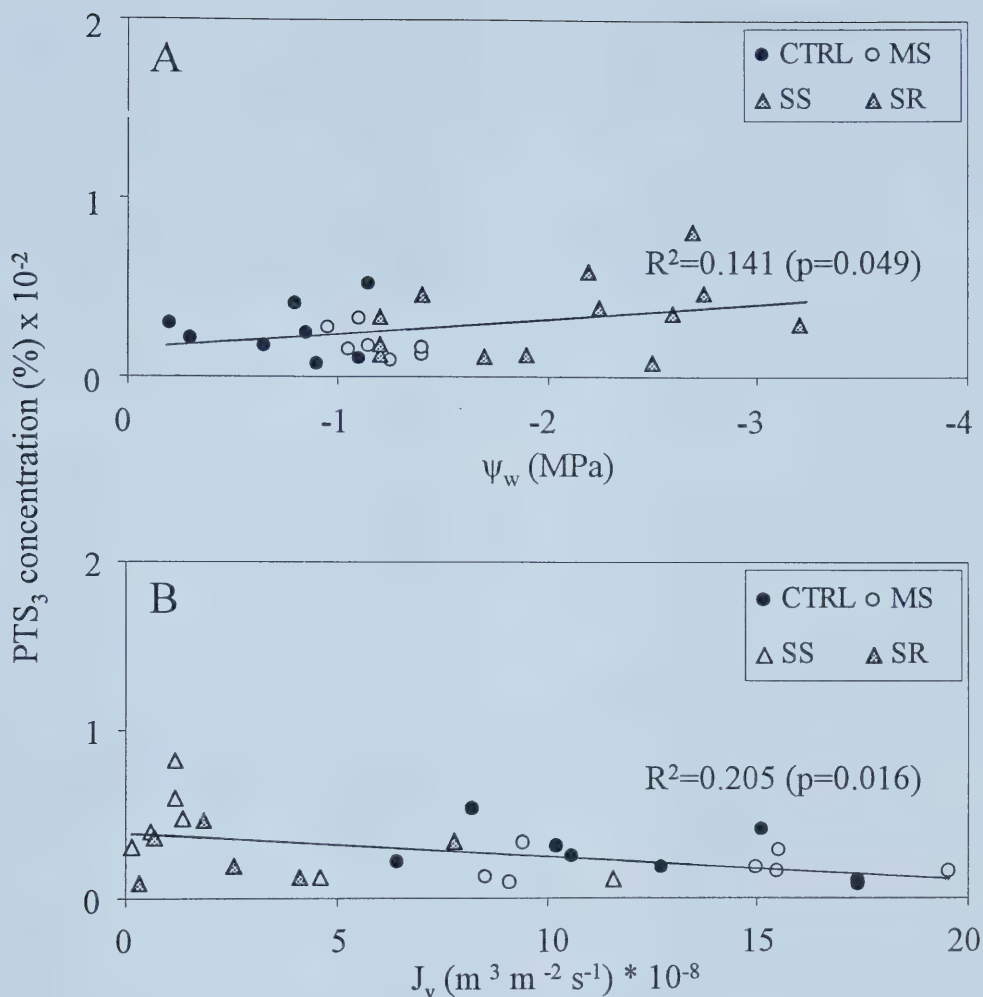


Figure 2.4. Relationship between water potential (ψ_w) and PTS₃ concentration in xylem exudate (A) and between root volume flow density (J_v) and PTS₃ concentration (B) for sand-grown aspen. Control (CTRL), mildly-stressed (MS), severely-stressed (SS), and stress-recovered (SR) treatments are shown. Linear regressions (A) and (B) are significant at $p \leq 0.05$.

Table 2.1. Apoplastic root flow measured before the addition of HgCl₂ (initial), and after the additions of 0.1 mM HgCl₂ and of 50 mM mercaptoethanol (ME) in control (CTRL), mildly-stressed (MS) severely-stressed (SS), and stress-recovered (SR) aspen seedlings. Apoplastic flow was estimated using the apoplastic PTS₃ dye at hydrostatic pressures of 1.0 MPa. Means \pm SE ($n_{\text{CTRL}}=8$, $n_{\text{MS}}=7$, $n_{\text{SS}}=7$, and $n_{\text{SR}}=6$) are shown. Differences in uppercase letters indicate significant ($p \leq 0.05$) differences only for initial values between treatments. Differences in lowercase letters indicate significant ($p \leq 0.05$) differences for each treatment between columns.

Apoplastic Flow (% Total)*			
Treatment	Initial	+ HgCl ₂	+ ME
CTRL	12.78 \pm 2.83 ^{ABa}	16.54 \pm 3.86 ^{ab}	19.36 \pm 4.39 ^b
MS	9.26 \pm 1.58 ^{Aa}	13.49 \pm 1.87 ^a	15.63 \pm 4.35 ^a
SS	20.13 \pm 4.82 ^{Ba}	13.37 \pm 4.09 ^b	17.27 \pm 3.91 ^{ab}
SR	12.84 \pm 3.34 ^{ABa}	9.14 \pm 3.98 ^a	14.38 \pm 5.62 ^a

* Apoplastic flow was calculated by dividing the PTS₃ concentration in xylem exudate (C_e) by the PTS₃ concentration in the original solution (C_s): $(C_e/C_s) \times 100$.

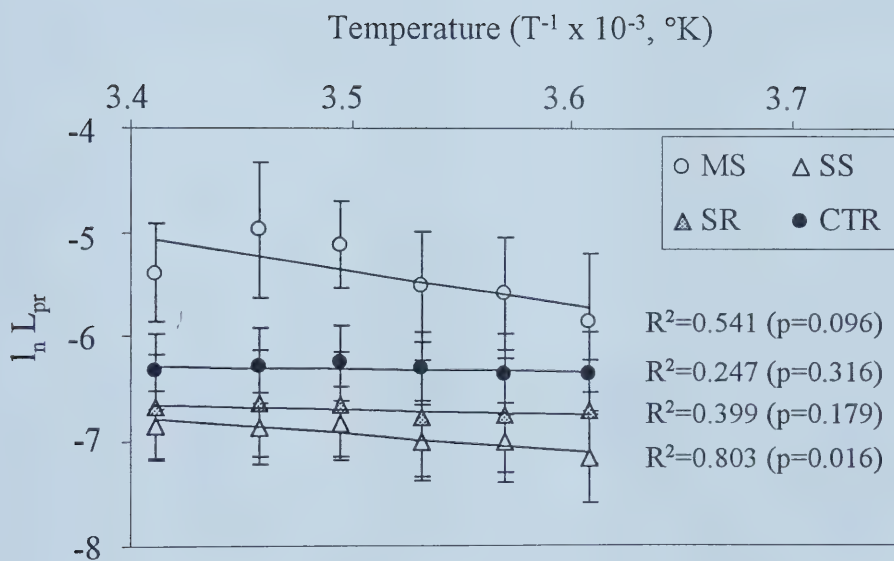


Figure 2.5. Descending Arrhenius plots of control (CTRL), mildly-stressed (MS), severely-stressed (SS), and stress-recovered (SR) aspen seedlings. Means \pm SE are shown ($n_{CTRL}=7$, $n_{MS}=7$, $n_{SS}=8$, $n_{SR}=8$).

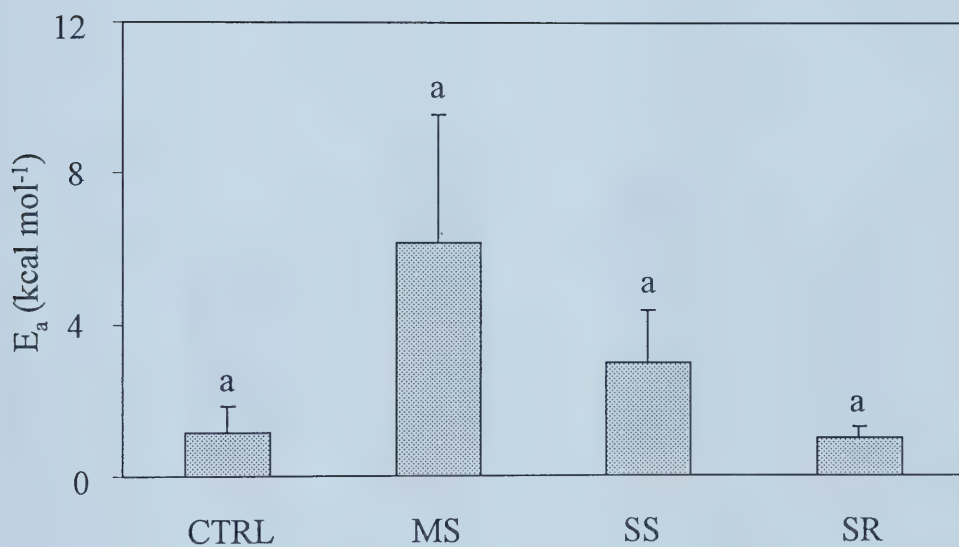


Figure 2.6. Activation energy (E_a) of control (CTRL), mildly-stressed (MS), severely-stressed (SS), and stress-recovered (SR) aspen. Bars with different letters are significantly different ($p \leq 0.05$). Means + SE are shown ($n_{\text{CTRL}}=7$, $n_{\text{MS}}=7$, $n_{\text{SS}}=8$, $n_{\text{SR}}=8$).

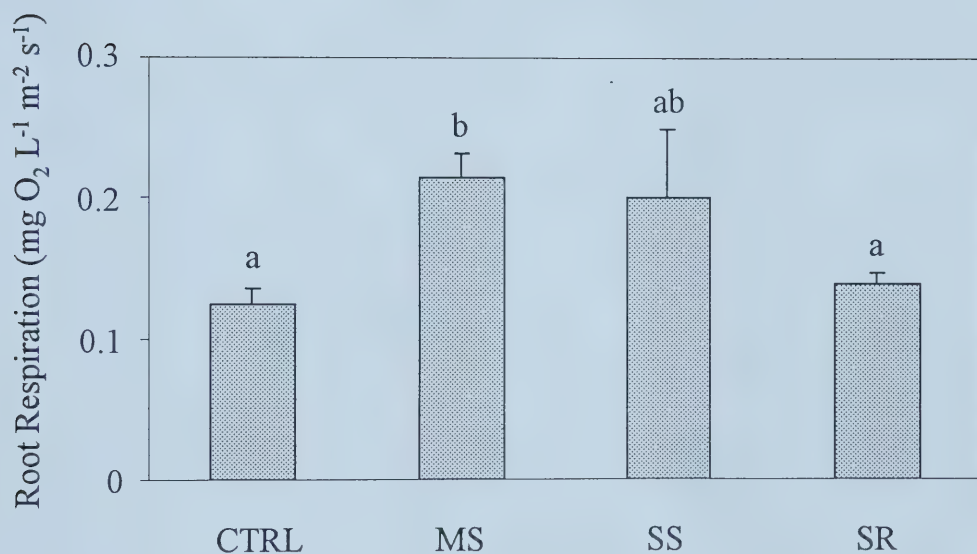


Figure 2.7. Root respiration rate of control (CTRL), mildly-stressed (MS), severely-stressed (SS), and stress-recovered (SR) aspen seedlings. Different letters indicate significant differences ($p \leq 0.05$). Means + SE are shown (all $n=6$).

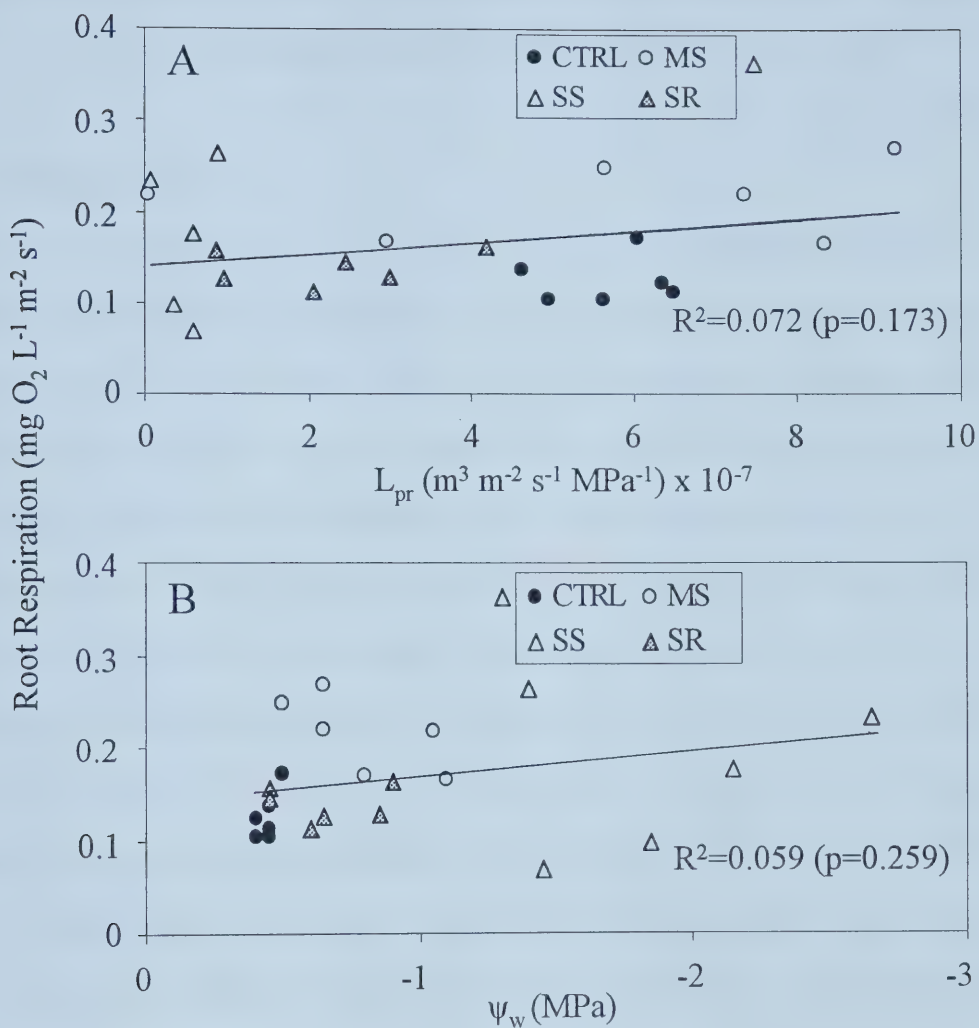


Figure 2.8. Relationship between root hydraulic conductivity (L_{pr}) and root respiration (A), and water potential (ψ_w) and root respiration (B). Control (CTRL), mildly-stressed (MS), severely-stressed (SS), and stress-recovered (SR) treatments are shown. Regressions for (A) and (B) are not significant at $p \leq 0.05$.

CHAPTER III

Root Water Flow Properties in Trembling Aspen (*Populus tremuloides*) Seedlings Grown and Subjected to Water Deficit Stress in Solution Culture

3.1 INTRODUCTION

Water deficit can have a major impact on the growth and function of woody plants (Boyer 1985, North and Nobel 1996, Lu and Neumann 1999). Numerous studies to date have examined the effects of water deficit stress (Markhart III 1984, Cruz et al. 1992, North and Nobel 1996, Dubrovsky et al. 1998, Lo Gullo et al. 1998, Nardini and Pitt 1999) and rehydration (North and Nobel 1992, 1996) on different plant species including woody plants (Tyree et al. 1992, Lo Gullo et al. 1998, Lu and Neumann 1999, Nardini and Pitt 1999, Sparks and Black 1999, Wakamiya-Noborio et al. 1999). It is necessary to understand the function of root systems in water uptake and their responses to environmental stresses in order to determine how plants are affected by water deficit stress. The regulation of water absorption is not clearly understood (Henzler et al. 1999).

Radial root water flux occurs through either the apoplastic (between protoplasts) or the cell-to-cell pathways (through plasmodesmata and across cell membranes) (Steudle et al. 1993, Steudle 1994, Steudle and Peterson 1998). Previous studies have indicated that the cell-to-cell pathway dominates under well-hydrated conditions (Steudle and Jeschke 1983, Smith and Nobel 1986, Steudle and Brinckmann 1989, Steudle et al. 1993). The proportion of apoplastic to cell-to-cell flow may change with water deficit stress due to increased cell wall thickenings and suberization of tissues (Steudle 1994, Tyerman et al. 1999) and with the presence of areas of resistance to root water flow such

as the endodermis and exodermis. The exodermis has been observed to be present in *Zea mays* when grown in aeroponics, but not hydroponics (Steudle and Peterson 1998, Tyerman et al. 1999). Some studies have attempted to quantify apoplastic flow with fluorescent, apoplastic tracer dyes such as PTS₃ (3-hydroxy-5, 8, 10-pyrenetrisulfonate) (Hanson et al. 1985, Moon et al. 1986, Skinner and Radin 1994, Kamaluddin and Zwiazek 2001) and Rhodamine B (Skinner and Radin 1994, Wan and Zwiazek 1999). Estimates of apoplastic flow using tracer dyes seem to considerably underestimate measurements of apoplastic flow conducted with a cell pressure probe (Peterson et al. 1981, Steudle and Peterson 1998, Zimmerman and Steudle 1998). However, apoplastic fluorescent dyes may be useful as an indicator of changes in the proportion of root water flow due to water deficit stress, even though they may not be useful for quantification of water flow.

Transmembrane water channels, called aquaporins (AQPs) may also be involved in metabolic regulation of root water flux through cell membranes (Daniels et al. 1994, Maurel et al. 1995, Johansson et al. 1998). AQPs facilitate water flux through cell membranes (Chrispeels and Maurel 1994, Steudle and Henzler 1995, Maurel et al. 1997, Niemietz and Tyerman 1997, Schäffner 1998). The activity of some AQPs can be determined by reversible blockage of AQPs with mercurial reagents with the subsequent addition of reducing reagents such as mercaptoethanol (Henzler and Steudle 1995, Maggio and Joly 1995, Carvajal et al. 1996, Shütz and Tyerman 1997, Maurel et al. 1997, Tyerman et al. 1999, Wan and Zwiazek 1999, Kamaluddin and Zwiazek 2001). Since there are indications that AQPs can become less active with reductions in apoplastic

water potential (Karmoker et al. 1991), AQPs may play a role in the regulation of root water flux when water is less available.

The methods used to measure the effects of water deficit stress may affect the results and how they can be applied to ecophysiological field situations and to other experiments. Several root water relations experiments to date have used plants grown in solution culture (Skinner and Radin 1994, Maggio and Joly 1995, Freundl et al. 1998, Zimmermann and Steudle 1998, Lu and Neumann 1999), aeroponics (Zimmermann and Steudle 1998, Freundl et al. 2000, Henzler et al. 1999), and soil or sand media (North and Nobel 1996, Lo Gullo et al. 1998, Henzler et al. 1999, Zhang et al. 1995, Cruz et al. 1992). Because the length of time taken to induce stress alters root morphological responses to water deficit (Dubrovsky et al. 1998), and because morphological differences may affect root response to soil drying, the growth medium and growth conditions used could have an effect on experimental findings.

The objectives of this study were to examine the effects of different levels of water deficit stress on the root water relations of aspen seedlings grown in solution culture, and to examine AQP activity and the proportion of apoplastic root water flow in stressed plants. Aspen was grown in solution culture because it produced different root anatomical characteristics than those of sand-grown aspen. It was hypothesized that, due to the differences in root anatomy, solution-grown aspen roots would possess relatively high cell-to-cell flow rates compared with sand-grown roots. We also expected that severe water deficit stress and the inhibition of AQP activity would reduce root hydraulic conductivity(L_{pr}) and increase the proportion of root apoplastic flow.

3.2 MATERIALS AND METHODS

3.2.1 Effect of water deficit stress on root water relations

3.2.1.1 Plant culture

Trembling aspen (*Populus tremuloides* Michx.) seedlings were germinated from seed collected in Edmonton, Alberta, Canada. Seeds were germinated for a week in Petri dishes on a filter paper with the addition of distilled water. One-week-old germinants were planted into styroblock trays filled with foam polymer plugs (Oasis potting medium, Beaver Plastics, Edmonton, AB). Germinants were grown in a controlled environment growth chamber with the following conditions: 22°C (day)/ 18°C (night); 60% RH; and a 16-h photoperiod with 285 $\mu\text{mol s}^{-1} \text{m}^{-2}$ photosynthetically active radiation (PAR) at plant height. Seedlings were bottom-watered daily, and bottom-fertilized with 0.1% 20-20-20 (N-P-K) fertilizer solution three times a week. Six-week-old seedlings were removed from the trays, the foam material was removed from their roots, and root systems were washed. Seedlings were placed into aerated solution culture, with roots immersed in 25% Hoagland's solution. The solution concentration was increased to 50% and to full-strength Hoagland's solution after one week, and nine days, respectively. Seedlings were grown in solution culture for one month, and solution was changed weekly. The mean dissolved O_2 concentration of the solution was $7.49 \pm 0.21 \text{ mg L}^{-1}$.

3.2.1.2 Water deficit stress treatments

Two stress levels were applied to 3.5-month-old solution culture-grown seedlings: mild stress (M), and severe stress (S). Two levels of water deficit stress were used to determine if there was a difference in the response of aspen to different levels of water deficit stress. Both water deficit stress treatments were induced by placing root systems of intact seedlings into a sealed, aerated, high-humidity (85-90% RH) container within the growth chamber. Mild stress was induced by maintaining seedling roots in the high-humidity chamber for approximately 17 hours until seedlings exhibited the first signs of leaf wilting. Severe stress was induced by maintaining seedling roots in the high-humidity chamber for approximately 18.5 hours until seedlings exhibited severe wilting. A control (CTRL) group consisted of seedlings in aerated solution culture. Unlike the water deficit stress experiments involving sand-grown aspen seedlings (Chapter II in this study), no stress recovery treatment was used.

3.2.1.3 Stomatal conductance and water potential

Stomatal conductance (g_s) was measured using a LI-1600 steady-state porometer (LI-COR, Lincoln, NE). One fully-expanded, uninjured leaf, from approximately mid-stem, was used for measurements for each plant.

Water potential (ψ_w) of the shoot, which was excised approximately 3 cm above the base of the root system, was measured using a Scholander pressure chamber (PMS Instruments, Corvallis, OR) as described by Wan et al. (1999).

3.2.1.4 Root hydraulic conductivity

In the first experiment, root hydraulic conductivity was measured using Scholander pressure chambers (PMS Instruments, Corvallis, OR) (Wan and Zwiazek 1999). Eight seedlings were used for each stress treatment and control. Measurements of g_s and ψ_w were taken for these seedlings to quantify the level of water deficit stress imposed, but measurements were not shown in this study. Detopped roots of solution culture-grown seedlings were immersed in distilled water at room temperature within the pressure chamber, while the cut section emerged through the pressure chamber lid. Root flow rate was quantified by attaching a graduated glass pipette to the cut section of each root system with a short section of rubber tubing, and water volume changes were averaged over the time period. Roots were gradually pressurized with air to 0.25 MPa, flow rate was allowed to stabilize, and steady-state flow rate was measured for 20 minutes. Pressure was then increased to 0.95 MPa, in 0.05 MPa increments. Root flow rate was allowed to stabilize at each pressure and steady-state flow rate was measured for 20 minutes at each pressure.

Roots were washed and surface-dried, then wrapped in aluminum foil, frozen in liquid nitrogen, and shattered into smaller fragments. Root surface areas were scanned using a computerized scanning program (Sigma Scan 4.0, Jandel Scientific, San Rafael, CA). Root volume flow density, J_v was calculated by expressing root flow rate over root surface area.

Root hydraulic conductivity, L_{pr} , ($\text{m}^3 \text{ m}^{-2} \text{ s}^{-1} \text{ MPa}^{-1}$) was calculated as the slope of the regression line of J_v plotted against hydrostatic pressure.

This experiment had been previously conducted three times with sand-grown aspen, but not with solution culture-grown aspen.

3.2.2 Effects of HgCl_2 and mercaptoethanol on root water relations

In the second experiment, Scholander pressure chambers were used to measure the root flow rate of seedlings, before and after the addition of HgCl_2 followed by 2-mercaptoethanol (ME). The number of seedlings used for control, M, and S groups were 7, 7, and 8, respectively. Measurements of g_s and ψ_w were taken for the seedlings, and the relationships between g_s and J_v and between J_v and ψ_w are shown in this study. Seedlings were removed from solution culture, and root systems were washed. To measure changes in the proportion of apoplastic water transport, a 0.02% (w/v) PTS_3 (3-hydroxy-5, 8, 10-pyrenetrisulfonate) solution was used in place of distilled water to measure root flow rates. PTS_3 is a nonionic fluorescent tracer dye that is used to detect apoplastic water transport (Moon et al. 1986, Skinner and Radin 1994). Root systems were immersed in 0.02% PTS_3 solution in the pressure chambers, and root flow rates were quantified using the method described above in section 3.2.1.4, by inducing a steady-state flow rate for a period of 60 minutes at 0.3 MPa.

Following measurements of original root flow rates, roots were removed from the pressure chambers, the solution was aerated, and HgCl_2 was added to the medium to produce a final concentration of 0.1 mM HgCl_2 . HgCl_2 has been shown to reduce movement of water through transmembrane water channels by physical blockage of water flow (Maurel et al. 1997, Maggio and Joly 1995). The medium was quickly stirred with a stirring bar, then roots and medium were placed back into the pressure chamber.

Pressure was gradually increased 0.3 MPa. Roots were pressurized for twenty minutes prior to resuming measurement of root flow rates. Root flow rate after the addition of HgCl_2 was measured for 60 minutes, and xylem exudate samples were collected for analysis of PTS_3 concentration.

Following root flow rate measurements in HgCl_2 solution, roots were removed from pressure chambers. Solutions were aerated and 2-mercaptoethanol (ME) was added to the medium to produce a final concentration of 50 mM ME. Pressure was gradually increased to 0.3 MPa. Root systems were pressurized for 20 minutes prior water flow rate measurements. Xylem exudate samples were collected after 60 minutes for PTS_3 concentration analysis. J_v values of treatment and control seedlings were calculated as described above. This experiment was previously conducted four times with sand-grown aspen. For three of those experiments, ME was not used to measure recovery of J_v from mercuric inhibition.

3.2.2.1 PTS_3 concentration analysis

PTS_3 concentrations in the xylem exudate samples were quantified using a Sequoia-Turner 450 fluorometer (Apple Scientific, Chesterland, OH) with a 405 nm excitation and 515 nm emission (Skinner and Radin1994). A standard curve was produced with known concentrations of PTS_3 . Exudate samples were diluted in distilled water to measure concentrations due to the sensitivity of the fluorometer, and actual xylem exudate concentrations were calculated using the dilution factors used. Apoplastic flow was estimated by dividing PTS_3 xylem concentrations by the concentration of PTS_3 used in the root solution medium (Kamaluddin and Zwiazek 2001).

3.2.3 Root respiration

In the third experiment, seedlings grown in solution culture were subjected to water deficit stress by removing roots from solution culture, gently washing the roots in tap water, and placing roots of intact seedlings into a sealed, aerated, high-humidity (90% RH) container at ambient temperature within the growth chamber. Six seedlings each were used for the stress treatment group and the control group. Only one level of water deficit stress was used in this experiment. Seedlings were exposed to water deficit stress for 21 hours. Control (CTRL) seedlings remained in aerated solution culture.

Root respiration rate was measured for stressed (S) and CTRL seedlings using a dissolved oxygen meter with a Clark-type electrode (YSI 5000, Yellow Springs Instruments, Yellow Springs, OH) to determine the rate of O₂ consumption over time. Root systems were immersed in distilled water within an airtight plexiglass container, with the electrode positioned at half-depth in the water. The container was placed into a shallow basin of water on a stirring plate, to minimize temperature effects due to the magnetic stirrer. While the water within the airtight container was stirred continuously, dissolved O₂ concentrations were measured over time at two-minute intervals for the first ten minutes, then at five-minutes intervals for the following ten minutes. The root respiration rate (mg O₂ L⁻¹ m⁻² s⁻¹) was calculated from the slope of the regression line of changes in O₂ concentration over time and expressed on a root area basis.

ψ_w , g_s , and J_v were measured as described above, with J_v measured at 0.3 MPa over a 30-minute period. All measurements are shown in this study.

The respiration experiment was previously repeated twice with sand-grown aspen.

3.2.4 Analysis of root morphology and anatomy

Non-stressed seedlings were taken for microscopic examination. A root with an intact root tip was selected from each root system, and free-hand cross sections were made of the roots from 0 to 60 mm from the root tip at 5 mm intervals. Root cross sections were placed in distilled water on glass microscope slides, and cover slips were placed over top of the specimens. Root sections were stained with 0.1% berberine hemisulphate for 1 h, then counterstained with 0.5% toluidine blue O for 45 minutes (Freundl et al. 2000). Excess berberine hemisulphate and toluidine blue stains were removed with the addition of distilled water. Sections were observed with an epifluorescence microscope (Nikon, Japan) using excitation/emission wavelengths of 365/395 nm (Wan and Zwiazek 2001).

3.2.5 Statistical analysis

Differences in the mean L_{pr} between the control, M, and S groups in the first experiment, were analyzed by univariate ANOVA using SPSS 10.0 (SPSS Inc., Chicago, IL) to determine if differences were significant. Univariate ANOVA was also used to analyze differences in percent initial apoplastic flow prior to the addition of $HgCl_2$ between the two treatment groups and the control group in the second experiment. Within each treatment or control group, differences between initial J_v , J_v after the addition of $HgCl_2$, and J_v after the addition of ME were analyzed using paired-t tests with SPSS. Similarly, differences between initial PTS_3 concentration, and PTS_3 after the subsequent additions of $HgCl_2$ and of ME were analyzed within each group using paired-t tests. Differences in ψ_w , g_s , J_v , and root respiration rate between the control and stressed

groups were analyzed by univariate ANOVA in the third experiment. Linear regression analyses ($y = mx + b$) were conducted on the relationships between g_s , ψ_w , and J_v in the first experiment, and on the relationships between PTS_3 concentration and ψ_w or J_v in the second experiment, to determine the significance of the relationship. For all statistical tests, the critical p-value was set at 0.05.

The statistical model used for univariate ANOVA using a completely randomized design was: $Y_{ij} = \mu + t_i + e_{ij}$

where Y_{ij} = value of individual observation (i = treatment, j = observation)

μ = overall mean

t_i = effect of i^{th} treatment (where i = level of water deficit stress)

e_{ij} = random error

3.3 RESULTS

3.3.1 Stomatal conductance, shoot water potential and root volume flow density

Stomatal conductance (g_s) and root volume flow density (J_v) both decreased ($p \leq 0.05$) with decreasing water potential (ψ_w) (Fig. 3.1A, B), which are the results from the experiments described in sections 3.2.1.3 and 3.2.2. ψ_w values for severely-stressed seedlings were between -1.5 and -3 MPa. In one repeated experiment involving solution culture-grown aspen, significant decreases in ψ_w and g_s were observed in mildly-stressed aspen (data not shown). J_v significantly ($p \leq 0.05$) increased with increasing g_s (Fig. 3.1C).

3.3.2 Root hydraulic conductivity

Results of root hydraulic conductivity are from the first experiment described in section 3.2.1.4. Root hydraulic conductivity (L_{pr}) did not change significantly with mild stress, but decreased significantly ($p \leq 0.05$) with severe stress (Fig.3.2). Two repeated experiments have shown a decrease in L_{pr} with water deficit stress in sand-grown aspen seedlings (data not shown).

3.3.3 Effects of $HgCl_2$ and mercaptoethanol on root water relations

Initial J_v values decreased significantly ($p \leq 0.05$) with mild stress, but the further decrease in severely-stressed seedlings was not significant compared to mildly-stressed seedlings (data not shown). Initial J_v values of control, mildly-stressed, and severely-stressed seedlings were 5.5, 2.8, and $1.8 \text{ m}^3 \text{ m}^{-2} \text{ s}^{-1} \times 10^{-8}$, respectively. In Figs. 3.3A and B, normalized values of J_v and PTS_3 concentration were used to adjust for differences in the actual initial J_v values. Because normalized J_v and PTS_3 values are used, there are no standard error bars for initial normalized J_v or PTS_3 .

Figs. 3.3A and B are the results of the second experiment described in section 3.2.2. In control and mildly-stressed seedlings, normalized J_v decreased significantly ($p \leq 0.05$) by 29.1% and 29.0%, respectively, after the addition of $HgCl_2$ (Fig. 3.3A). The subsequent addition of ME to the $HgCl_2$ solution caused further significant decreases in normalized J_v of 45.7% and 22.6%, respectively, compared to the J_v values of the roots in $HgCl_2$ solution (Fig. 3.3A). In severely-stressed seedlings, normalized J_v decreased by 2.6% after the addition of $HgCl_2$, but the decrease was not significant (Fig. 3.3A). The

addition of ME to the HgCl_2 solution produced a further significant ($p \leq 0.05$) decrease of 12.3% (Fig. 3.3A).

3.3.4 PTS_3 concentration in xylem exudate

Initial PTS_3 concentrations of solution culture-grown aspen significantly ($p \leq 0.05$) decreased in mildly-stressed seedlings, relative to controls, but severely-stressed seedlings were not significantly different from controls or mildly-stressed seedlings (data not shown). Initial PTS_3 concentrations of control, mildly-stressed, and severely-stressed seedlings were 1.8, 0.49, and $2.3\% \times 10^{-3}$, respectively. In control seedlings, normalized PTS_3 concentrations increased by 59.0% after adding HgCl_2 and by a further 58.4% after the subsequent addition of ME to the HgCl_2 solution, but only the latter increase was significant ($p \leq 0.05$). Mildly-stressed seedlings significantly ($p \leq 0.05$) increased by 85.6% and by a further 119.3% with the additions of HgCl_2 and ME, respectively. Severely-stressed seedlings showed a non-significant decrease of 1.9% with HgCl_2 and a significant increase of 76.0% with ME.

Increasing trends for apoplastic flow calculated for solution culture-grown seedlings were similar to those of PTS_3 concentrations for controls and stress treatments (Table 3.1).

PTS_3 concentration increased with decreasing ψ_w (Fig. 3.4A) and with decreasing J_v (Fig. 3.4B), but the linear regression was only significant ($p \leq 0.05$) for the former.

3.3.5 Root respiration

Root respiration results were from the third experiment described in section 3.2.3. In solution culture-grown aspen, root respiration increased significantly ($p \leq 0.05$) in stressed seedlings (Table 3.2). The linear regression between g_s and respiration was significant ($p \leq 0.05$) and showed that respiration increased with decreasing g_s (data not shown). Although respiration increased with decreasing ψ_w and decreasing J_v , linear regressions between respiration and ψ_w , and between respiration and J_v , were not significant (data not shown).

3.3.6 Root morphology and anatomy

Roots of solution culture-grown aspen were morphologically different from those of aspen grown in sand. Roots of sand-grown aspen had many lateral roots and fine roots (Fig.3.5a). Solution culture-grown aspen had straighter roots and fewer lateral roots compared to sand-grown aspen, and fine roots were absent (Fig.3.5b).

Roots of sand-grown aspen appeared to possess an endodermis surrounding the stele, and a discontinuous exodermis in the region just below the epidermis in root cross-sections stained with berberine hemisulphate (Figure 3.5c). The endodermis and exodermis appeared to extend along the edges of the lateral root. The exodermis was first seen at 5 mm from the root tip, indicated by the fluorescing suberin lamellae between the radial walls, but did not appear to be continuous (data not shown). Beyond 45 mm from the root tip, the exodermis was no longer visible. In cross-sections of solution culture-grown roots, a definite endodermis appeared at approximately 10 mm from the root tip, but no exodermis was observed from 0-60 mm from the root tip (Figure 3.5d).

3.4 DISCUSSION

3.4.1 *Effect of water deficit stress on root water relations*

In the present study, severe water deficit in solution culture-grown aspen resulted in significant decreases in g_s , ψ_w , J_v , and L_{pr} . The significant relationships between g_s , ψ_w , and J_v in the present study (Figs. 3.1A-C) indicate that these parameters are interrelated. According to the cohesion-tension theory, the rate of g_s determines the rate of root water flow because of the xylem tension (Steudle 2001). Therefore, low ψ_w in stressed seedlings could have induced stomatal closure to prevent excessive water loss (Pierce and Raschke 1980, Schulze 1993), which could have prevented some xylem cavitation (Sperry et al. 1993, Kozlowski and Pallardy 1997), and could have reduced root water flow rates as well.

In the present study, L_{pr} increased in mildly-stressed seedlings with a decrease in ψ_w compared to controls, although the increase in L_{pr} was not significant (Fig. 3.2). Osmotic adjustment of guard cells can occur in response to water stress-induced decreases in tissue ψ_w , allowing guard cells to maintain turgor during initial water loss (Parker et al. 1982, Roden et al. 1990). Therefore, osmotic adjustment could have maintained g_s and root water flux (Steudle and Peterson 1998, Henzler et al. 1999) during mild stress. Similarly, osmotic adjustment in the roots could have prevented a decrease in J_v and L_{pr} with moderate water deficits, temporarily preventing increases in tissue resistance to water flux that are associated with water deficit.

In the present study, reduction of L_{pr} (Fig. 3.2) and J_v (data not shown) occurred due to severe stress. The low ψ_w in severely-stressed seedlings (data not shown) might

indicate that there were some xylem embolisms present, as they can result in a reduction of J_v and L_{pr} (Nardini and Pitt 1999, Tyree and Sperry 1988, 1989, Sperry et al. 1993), stomatal closure, and leaf wilting (Boyer 1985). The amount of time required to produce severe stress and the extent of xylem embolisms required to significantly reduce root water flux are dependent on the drought resistance of the plant species in question (Sparks and Black 1999, Wakamiya-Noborio et al. 1999). Although pressure was used to induce a steady-state flow to measure J_v and L_{pr} , and lower pressures than those used in the present study have been used to flush embolisms from *Acer* stems (Sperry et al. 1988), it is possible that not all embolisms present were flushed from the xylem vessels, as the xylem exudate expressed from stressed seedlings contained small air bubbles.

Anatomical and morphological changes can occur within roots as a result of exposure to water deficit stress. Root tissues such as the endodermis, exodermis, and root tips can become thickened and suberized (Kramer 1983, Lo Gullo et al. 1998), reducing water flux into the root during stress (Cruz et al. 1992, North and Nobel 1996). Lateral root formation can also occur during water deficit, particularly during rapid water deficit stress, and can also help with stress recovery (Zhang et al. 1995, Dubrovsky et al. 1998, Lo Gullo et al. 1998, Nardini and Pitt 1999). However, comparative microscopy of water deficit-stressed and control roots would have been necessary to determine if tissue resistance or lateral root formation was a major contributing factor to the reduction of J_v and L_{pr} found in the present study.

Several studies, many of which used non-woody plant species, have shown that water deficits result in decreases of stomatal conductance (g_s), water potential (ψ_w), and hydraulic conductance (K_r) or hydraulic conductivity (L_p) (Cruz et al. 1992, Tyree et al.

1992, Sperry et al. 1993, Zhang et al. 1995, Dubrovsky et al. 1998, Lo Gullo et al. 1998, Lu and Neumann 1999, Nardini and Pitt 1999, Sparks and Black 1999, Wakamiya-Noborio et al. 1999, Martre et al. 2001). Some studies show that L_{pr} can recover with rewatering (North and Nobel 1996, Lo Gullo et al. 1998, Martre et al. 2001), although only Lo Gullo et al. (1998) used a woody plant species, while the other studies used succulent species. Severely-stressed plants may require several days to recover (Kramer 1950, Martre et al. 2001).

J_v and L_{pr} values in the present study were similar but slightly higher in comparison with experiments using solution culture-grown trembling aspen (Wan and Zwiazek 1999, 2001, Wan et al. 2001) and dogwood (*Cornus stolonifera*) (Kamaluddin and Zwiazek 2001). However, g_s values for solution culture-grown control seedlings (Fig. 3.1A) in this study were considerably higher than in these previous studies, and in repeated experiments that were not shown in this study. The high g_s values may have been due to higher relative humidity in the present study. Aspen showed up to a three-fold increase in g_s with an increase of 45% to 60% RH, due to growth chamber renovations (discussed in Chapter V) which could indicate that aspen appear to be rather sensitive to humidity changes.

It should be noted that inducing precisely the same level of mild and severe water deficit stress in each experiment was not a possibility, therefore results from repeated experiments tended to vary between experiments. Repeated experiments, therefore, often showed similar trends in the effects of water deficit stress and in the relationships between the various water relations parameters. These differences or relationships were not always significant due to variability in the response of individual seedlings to water

deficit stress, and because of the difficulty in reproducing the same level of mild and severe water deficit stress. Aspen grown in sand seemed to respond variably to different levels of water deficit stress (Chapter II in this study) Variability in response has also been observed by Wakamiya-Noborio et al. (1999). As a result, the data shown in the present study is representative of the effects of water deficit stress on trembling aspen seedlings based on repeated experiments, although data from repeated experiments were not shown.

3.4.2 Root morphology and anatomy

The present study showed that root morphology and anatomy of solution culture-grown aspen differed from sand-grown aspen. Solution culture-grown aspen had a low number of lateral roots, a larger root diameter, and fine roots were absent (Fig. 3.5b), which may have resulted in a low root surface area compared to sand-grown aspen roots. A large surface area for water and mineral absorption is not necessary for solution culture-grown aspen because water availability is not a limiting factor for seedling growth in solution culture. The fact that water was not limiting may also have been indicated by the higher g_s in solution culture-grown aspen, compared with sand-grown aspen (Chapter II), which suggests that there was little ψ_w -related root resistance to limit hydraulically-driven uptake of water.

In comparison, sand-grown aspen roots were highly-branched, had a smaller root diameter, and may have had a larger root surface area for absorbing water than solution culture-grown aspen (Fig. 3.5a). Sand-grown control aspen had higher L_{pr} values (Chapter II), compared to solution culture-grown controls, which may have been partly

due to root morphological differences. Root systems that penetrate deeper into the soil and that are more highly branched are better able to access and absorb water, and better able to cope with moderate amounts of water deficit (Hinckley et al. 1981, Nardini and Pitt 1999). Highly-branched and large root systems are beneficial in soil medium to increase the absorptive capabilities of root systems, particularly when water is a limiting factor. The root morphology of sand-grown aspen is likely closer to the root morphology of field-grown aspen than is the solution culture-grown aspen.

Due to differences in the induction of water deficit stress, which was rapid for solution culture-grown aspen and more gradual for sand-grown aspen (Chapter II), results from water deficit stress experiments between sand- and solution culture-grown aspen could not be directly compared. During gradual water deficit stress, all water relations parameters decrease concurrently because each parameter is affected by the other parameters. During rapid stress, certain parameters such as g_s , J_v , and L_{pr} , may be more severely affected by water deficit stress because immediate tissue dehydration may have a localized effect on tissue ψ_w and on localized metabolism. However, parameters affected by whole-plant metabolic processes, such as AQP activity and increased tissue suberization would not necessarily be affected by rapid stress. Therefore the method of water deficit stress likely had an effect on the significance of the relationships between g_s , ψ_w , J_v , and L_{pr} .

3.4.3 Effects of $HgCl_2$ and mercaptoethanol on root water relations

Several studies have shown mercuric inhibition of AQPs that resulted in reductions in root water flux (Maggio and Joly 1995, Carvajal et al. 1996, Shütz and

Tyerman 1997, Lu and Neumann 1999, Zhang and Tyerman 1999). Mercuric inhibition has resulted in decreases of 47% of J_v in aspen (Wan and Zwiazek 1999), and of 46-52% of root water flow rate (Q_v) in dogwood (Kamaluddin and Zwiazek 2001) under similar measurement conditions and with pressures of 0.3 MPa.

In the present study, $HgCl_2$ resulted in a significant decline in J_v solution culture-grown aspen control seedlings (Fig. 3.3A), although inhibition rates were not as high as in the studies by Wan and Zwiazek (1999) and Kamaluddin and Zwiazek (2001). However, mercuric inhibition in the present study was greater than that for sand-grown aspen, even though higher pressures were used for sand-grown aspen (Chapter II). The lack of an exodermis in solution culture-grown aspen, which was observed in this study (Fig. 3.5d) and by Wan and Zwiazek (2001), could be the reason for higher mercuric inhibition compared to sand-grown aspen which does possess an exodermis. It is known that $HgCl_2$ can diffuse through cell membranes (Tyerman et al. 1999). The present study indicates that low concentrations of $HgCl_2$ were able to penetrate aspen root systems at low pressure, unlike the higher pressures required for mercurial inhibition of AQPs in sand-grown aspen (Chapter II). The results indicate that AQPs within aspen roots are mercury-sensitive. The fact that the same concentrations of $HgCl_2$ used for a similar length of time did not significantly reduce root respiration (Wan and Zwiazek 1999) is evidence that the reduction in root water flux due to the addition of $HgCl_2$ is not due to metabolic effects of $HgCl_2$. Therefore the reduction of J_v in the present study was likely due to inhibition of AQP activity. The results also suggest that, because mercuric inhibition resulted in a significant decrease in J_v , AQP activity can have a significant

effect on J_v , and therefore AQP may have a significant role in regulating cell-to-cell root water flow.

The current study indicates that AQPs may be deactivated as a result of water deficit stress. Activity of some AQPs can be metabolically regulated by enzymatic phosphorylation (active state) and dephosphorylation (inactive state), which allows for some regulation of cell-to-cell water movement (Daniels et al. 1994, Maurel et al. 1995, Johansson et al. 1998). Previous experiments suggest that AQPs have some regulatory role regarding root water flux, since genetic expression of AQPs has been highly correlated with changes in L_p (Henzler et al. 1999), and reductions in AQP activity have occurred with decreases in apoplastic water potential (Karmoker et al. 1991). The present study shows that, although significant mercuric inhibition occurred in control seedlings, there was no significant mercuric inhibition in severely-stressed seedlings (Fig. 3.3A). If AQPs are already deactivated as a result of water deficit stress, which may be a mechanism to help reduce water loss from roots, then further additions of $HgCl_2$ will not result in further blockage of AQPs. AQP deactivation would not only prevent water loss from roots but would also prevent a substantial proportion of water movement into roots. The results from the present study suggest that AQPs may be able to regulate root water flow under water deficit stress.

Previous studies in non-woody plants have also shown a lack of mercuric inhibition in less metabolically active AQPs (Zhang and Tyerman 1999) and during water deficits (Martre et al. 2001). These studies confirm that AQPs could be deactivated under conditions of stress in woody plants as well.

The current study shows that mercaptoethanol (ME) did not reverse mercuric inhibition of AQPs. Although 50 mM is the concentration of ME which has successfully reversed mercuric inhibition of AQPs according to the literature (Tyerman et al. 1999, Wan and Zwiazek 1999), Kamaluddin and Zwiazek (2001) showed only a partial reversal of HgCl_2 with 50 mM ME. ME is a toxic reagent and can negatively affect metabolic processes. In the present study, ME caused a significant decrease in J_v in control seedlings but did not produce a significant reduction in J_v of mildly- and severely-stressed seedlings (Fig. 3.3A). Since this study was completed, experiments have been conducted that suggest 5-20 mM ME is the optimal concentration for reversal of mercuric inhibition, and that 50 mM reduces root water flow via metabolic inhibition (Kamaluddin, unpublished results). Therefore, the lack of reversal of mercuric inhibition in the present study is likely the result of an ME concentration that is too high.

The lack of an exodermis in solution culture-grown roots may explain the slightly greater mercuric inhibition of AQPs compared with sand-grown seedlings (Chapter II). The exodermis in sand-grown seedlings likely prevented HgCl_2 from moving into roots at low pressures (Chapter II).

Although the role of the exodermis as an area of resistance to root water flux remains unclear (Steudle and Peterson 1998), it is possible that its presence provides some resistance to moderate water deficit stress by preventing water loss from roots back into soil by osmotic forces when the transpirational gradient is low (Passioura and Tanner 1985, Henzler et al. 1999, Tyerman et al. 1999). It has been observed that non-woody plants grown in hydroponics have lacked an exodermis, only to form an exodermis when grown in soil (Peterson 1988, Freundl et al. 1998, Zimmerman and Steudle 1998).

Therefore, solution culture-grown plants that lack an exodermis may in fact be more susceptible to water deficits when they are removed from solution culture. The fact that the exodermis was not present in aspen grown in solution culture, but was present in aspen grown in sand in the present study, is similar to the presence or lack of an exodermis depending on the growing conditions of *Zea mays* (Zimmerman et al. 2000). The exodermis may therefore have a role in providing some water deficit stress resistance to seedlings.

3.4.4 PTS_3 concentration in xylem exudate

PTS_3 has been used to estimate apoplastic flow in several experiments, with estimates that less than 2% of water flux is through the apoplastic pathway (Hanson et al. 1985, Moon et al. 1986, Varney et al. 1993, Skinner and Radin 1994, Wan and Zwiazek 1999, Kamaluddin and Zwiazek 2001). In some studies, such estimates substantially underestimated apoplastic measurements obtained from the cell pressure probe (Peterson et al. 1981, Skinner and Radin 1994, Zimmerman and Steudle 1998).

The present study suggests apoplastic flow may increase with AQP deactivation. In control and mildly-stressed solution culture-grown aspen, PTS_3 concentrations increased concurrently (Fig. 3.3B) with decreases in J_v (Fig. 3.3A) following the additions of $HgCl_2$ and of ME. The increase in PTS_3 concentration corresponded to the mercuric inhibition of AQPs. PTS_3 concentration was used as an estimate of apoplastic flow (Table 3.1), therefore, an increase in PTS_3 concentration indicates an increase in apoplastic flow.

The relationship between AQP deactivation and apoplastic flow is also supported by the lack of increase in apoplastic flow following the addition of HgCl_2 in stressed seedlings. PTS_3 did not show a significant increase in concentration with the addition of HgCl_2 (Fig. 3.3B), which corresponds to the lack of AQP deactivation following the addition of HgCl_2 (Fig. 3.3A), in severely-stressed seedlings. The lack of an increase in apoplastic flow supports the evidence that AQPs have been deactivated in severely-stressed seedlings, indicating that AQPs may have a role in regulating root water flow during water deficit stress. Apoplastic flow seemed to increase with water deficit, indicated by the relationship between PTS_3 concentration and ψ_w (Fig.3.4A). This trend was also observed for sand-grown aspen (Chapter II).

Based on these results of AQP activity and apoplastic flow, AQP deactivation may have resulted in a reduction in overall root water flow, with an increasing proportion of that root water flow occurring via the apoplastic pathway. The results also suggest that apoplastic flow may be more important in water deficit-stressed seedlings, because AQPs seem to be able to regulate a substantial portion of total root water flow. Apoplastic flow has been shown to play an important role in root water flux during water deficit stress and rehydration (Robards et al.1979, North and Nobel 1996).

The values for apoplastic flow calculated from PTS_3 concentrations are considerably higher than 2% apoplastic flow or less obtained in the studies mentioned above, and in studies involving dogwood (Kamaluddin and Zwiazek 2001) and aspen seedlings (Wan and Zwiazek 1999). However, aspen used in the present study were at least three months younger than plants used in previous experiments. Younger aspen have a greater proportion of young, unsuberized, fine roots than older seedlings.

Therefore, the higher apoplastic flow rates (Table 3.1) could be attributed to the higher proportion of lateral roots, or perhaps the lack of suberized cell walls.

Several studies (Peterson et al. 1981, Hanson et al. 1985, Skinner and Radin 1994, Zimmerman and Steudle 1998) have indicated that the use of apoplastic tracer dyes are not reliable, in part because the dye molecules are considerably larger than water and may not travel through exactly the same pathways as water. Although PTS_3 molecules are confined to the apoplast, some apoplastic spaces may not allow PTS_3 to pass.

Root morphology and anatomy may contribute to an increase or decrease in apoplastic flow, and it was expected that solution culture-grown aspen would have a lower percentage of apoplastic flow compared to sand-grown aspen because solution culture-grown roots lack an exodermis (Fig. 3.5d). Sand-grown roots have an exodermis (Fig. 3.5c), but had higher percent apoplastic flow (Chapter II). The exodermis, with its Casparian band and hydrophobic suberin lamellae, may force water to move through the cell-to-cell pathway. Increases in tissue suberization or the presence of areas of resistance to water flux may change the proportion of apoplastic flow through roots (Tyerman et al. 1999). However, lateral roots, which break through the endodermis, have been shown to provide a means for water and such dyes to bypass the endodermis until the endodermis in lateral roots becomes mature (Dumbroff and Peirson 1971, North and Nobel 1996, Steudle and Peterson 1998). In the present study, microscopy showed that the endodermis, and the exodermis in sand-grown roots, were not continuous through the lateral root (Fig. 3.5), which could have produced gaps in the endodermis and exodermis for the entrance of PTS_3 . Disturbance or damage to roots, such as that caused by removal of roots from growth medium, can also substantially increase apoplastic flow, allowing

water to enter via the lateral roots (Moon et al. 1986, Skinner and Radin 1994, North and Nobel 1996). Root movement in solution due to currents produced by the aeration system may also be considered disturbance. The fact that sand-grown aspen seedlings had higher apoplastic flow rates may be due to a higher level of disturbance and possibly damage to sand-grown roots upon removal from sand, resulting in artificially high percent apoplastic flow.

The present findings from this study are consistent with previous studies where a lack of effect of HgCl_2 on the severely-stressed seedlings and on less metabolically active plants was observed (Karmoker et al. 1991, Zhang and Tyerman 1999, Martre et al. 2001).

3.4.5 Root respiration

Although root respiration rate significantly increased with water deficit stress, g_s , ψ_w , and J_v showed significant decreases (Table 3.2). Increases in respiration with increasing water deficit stress were also observed in sand-grown aspen (Chapter II). It was expected that metabolic activity, of which respiration rate is an indicator, would decrease with water deficit stress. Respiration has been shown to decrease with NaN_3 , which can cause reductions in metabolic activity, and coincided with reductions in AQP activity (Kamaluddin and Zwiazek 2001). Respiration also decreased with high concentrations of HgCl_2 (Wan and Zwiazek 1999), which were higher than the concentration used in this study.

The increase in respiration may be due to changes in AQP expression or activity. Because AQPs can be metabolically regulated, it is possible that the increase in

respiration was indicative of an increase in metabolic activity required for AQP deactivation and, therefore, regulation of root water flow. However, the results from this study are not sufficient to confirm the relationship between root respiration rate and AQP activity.

The increases in respiration in the present study may also indicate that metabolic activity of water deficit-stress seedlings was enhanced by the addition of water during respiration measurements, because the present study showed that AQP activity was reduced in severely-stressed seedlings. If roots undergo osmotic adjustment during water deficit stress, then the accumulation of metabolites in roots could result in a greater increase in respiration in water deficit-stressed seedlings once water became available. If this is the case, then the increase in respiration is due to rehydration effects and not due to an increase in metabolic activity at the whole-root system level as a result of water deficit. Immediate localized metabolic responses to rehydration would not necessarily have resulted in an immediate increase in J_v in the present study.

In conclusion, solution culture-grown aspen were morphologically and anatomically different compared to sand-grown aspen. Solution culture-grown root systems were characterized as having few lateral roots, and lacking an exodermis. Such morphological and anatomical adaptations likely resulted from growing in solution culture where water was not lacking from their environment and may, therefore, render solution culture-grown aspen more susceptible to moderate water deficits when removed from solution culture, compared to sand-grown aspen. The exodermis may be a feature that provides resistance to water deficit stress in root systems.

Mild and severe water deficit stress caused decreases in g_s , ψ_w , J_v , and L_{pr} in solution culture-grown aspen. The study suggests water deficit stress resulted in AQP deactivation. Increased percent apoplastic flow, and lack of mercuric inhibition of AQPs were the result of AQP deactivation in stressed seedlings. Increased respiration due to water deficit stress may have been due to changes in AQP expression or AQP activity, but further experiments would be required to confirm this relationship. AQPs, therefore, seem to play a significant role in cell-to-cell flow, indicated by the increase in apoplastic flow during mercuric inhibition. Apoplastic flow may become an important pathway for root water flow in water deficit-stressed seedlings. AQPs also seem to be able to regulate root water flow during water deficit stress through deactivation and reduction of water flow. AQP deactivation may be necessary for seedlings to prevent excess water loss through the roots during water deficit. This study confirms some of the observations made from AQP activity and water deficit stress research conducted using non-woody plant species. Because very little is known about the effects of different levels of water deficit stress or AQP activity in woody plants, more research is needed to further out understanding about root water flow regulation and the role of AQPs when woody plants are exposed to environmental stresses.

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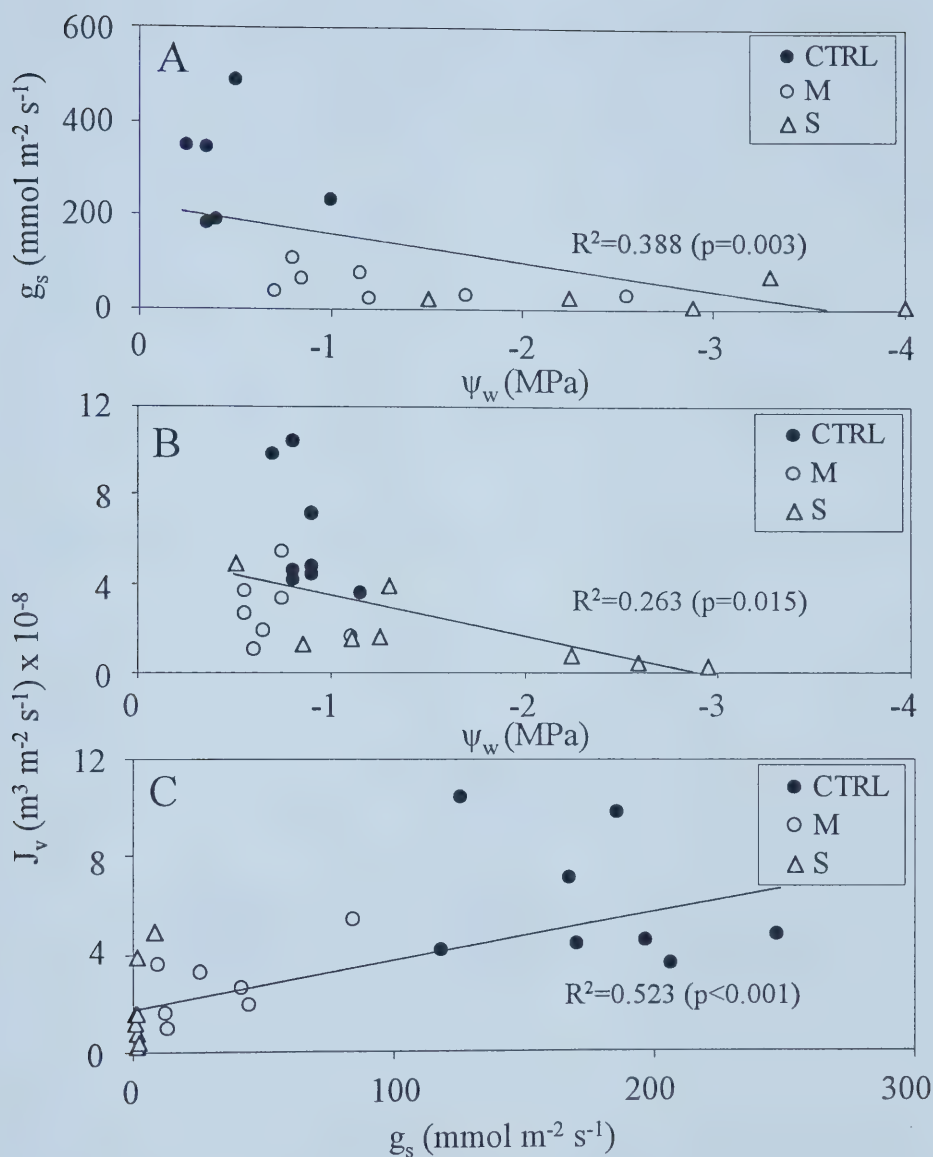


Figure 3.1. Relationship between water potential (ψ_w) and stomatal conductance (g_s) (A), and root volume flow density (J_v) (B), and g_s and J_v (C) for aspen seedlings grown in solution culture. Control (CTRL), mildly-stressed (M), and severely-stressed (S) treatments are shown. Linear regressions are significant at $p \leq 0.05$.

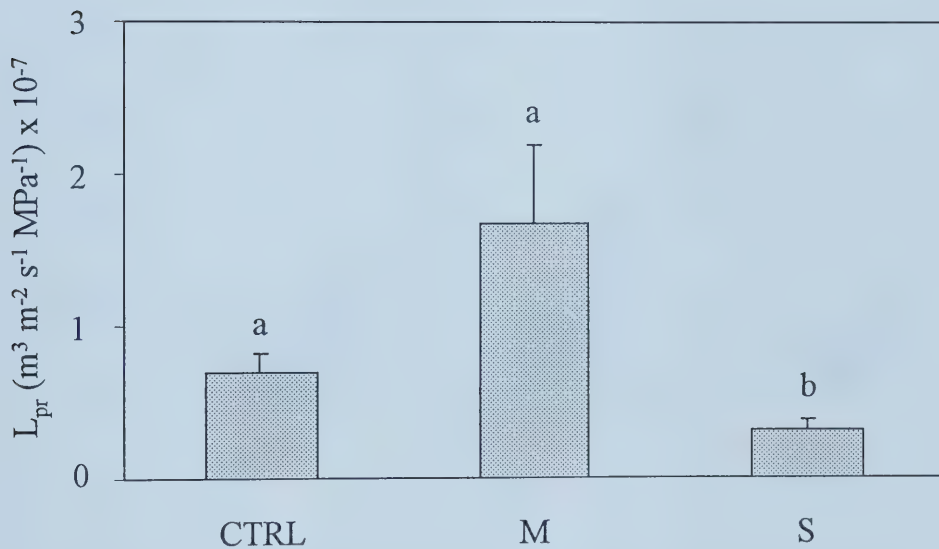


Figure 3.2. Root hydraulic conductivity (L_{pr}) of aspen grown in solution culture. Control (CTRL), mild stress (M), and severe stress (S) treatments are shown. Bars with different letters are significantly different ($p \leq 0.05$). Means + SE are shown (all $n=8$).

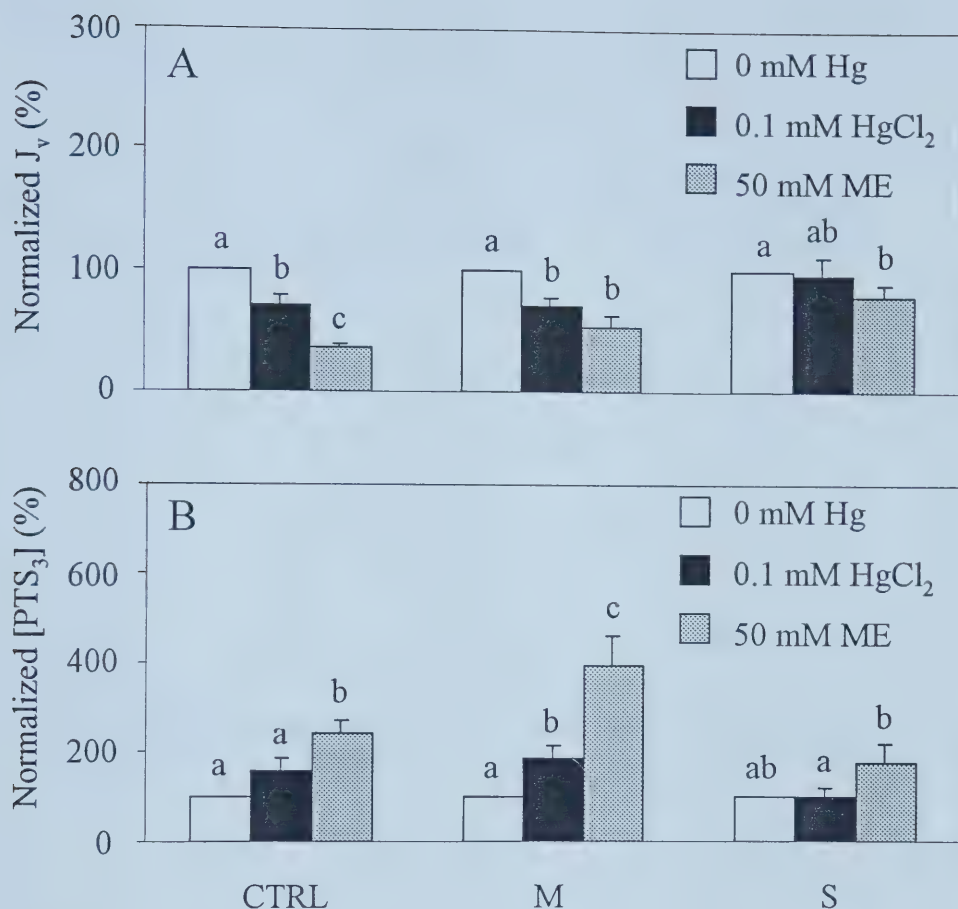


Figure 3.3. Normalized root volume flow density (J_v) (A) and normalized PTS₃ concentration (B) of control (CTRL), mildly-stressed (M), and severely-stressed (S) aspen. J_v and PTS₃ was measured before treatment of roots with HgCl₂, and after the addition of 0.1 mM HgCl₂ and of 50 mM 2-mercaptoethanol (ME). Aspen were grown in solution culture. Bars with different letters within each treatment are significantly different ($p \leq 0.05$). Means + SE are shown ($n_{\text{CTRL}}=7$, $n_{\text{M}}=7$, $n_{\text{S}}=8$).

Table 3.1. Percent initial apoplastic root flow before the addition of HgCl_2 , and after the additions of 0.1 mM HgCl_2 and of 50 mM mercaptoethanol (ME) in solution culture-grown aspen seedlings. Apoplastic flow was estimated using the apoplastic PTS_3 dye at hydrostatic pressures of 0.3 MPa. Means \pm SE of control (CTRL, n=7), mildly-stressed (M, n=7), and severely-stressed (S, n=8) treatments are shown. Differences in uppercase letters indicate significant ($p \leq 0.05$) differences only for initial values between treatments. Differences in lowercase letters indicate significant differences ($p \leq 0.05$) within treatments between columns.

Apoplastic Flow (% Total)*			
Treatment	Initial	After HgCl_2	After ME
CTRL	$9.05 \pm 2.03^{\text{Aa}}$	$13.31 \pm 3.20^{\text{b}}$	$20.28 \pm 4.68^{\text{c}}$
M	$2.43 \pm 0.66^{\text{Ba}}$	$4.16 \pm 0.88^{\text{b}}$	$7.72 \pm 0.97^{\text{c}}$
S	$11.71 \pm 4.91^{\text{ABab}}$	$8.81 \pm 3.10^{\text{a}}$	$15.04 \pm 5.26^{\text{b}}$

* Apoplastic flow was calculated by dividing the PTS_3 concentration in xylem exudate (C_e) by the PTS_3 concentration in the original solution (C_s): $(C_e/C_s) \times 100$.

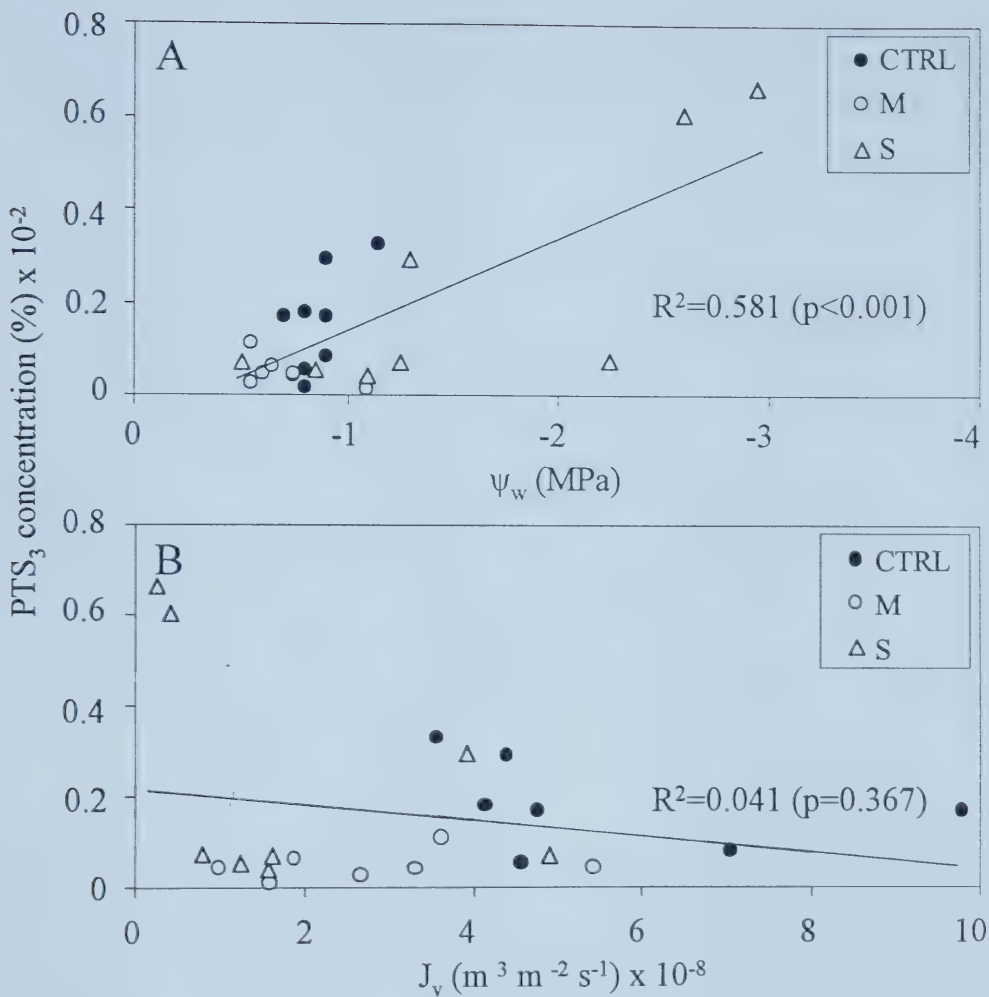
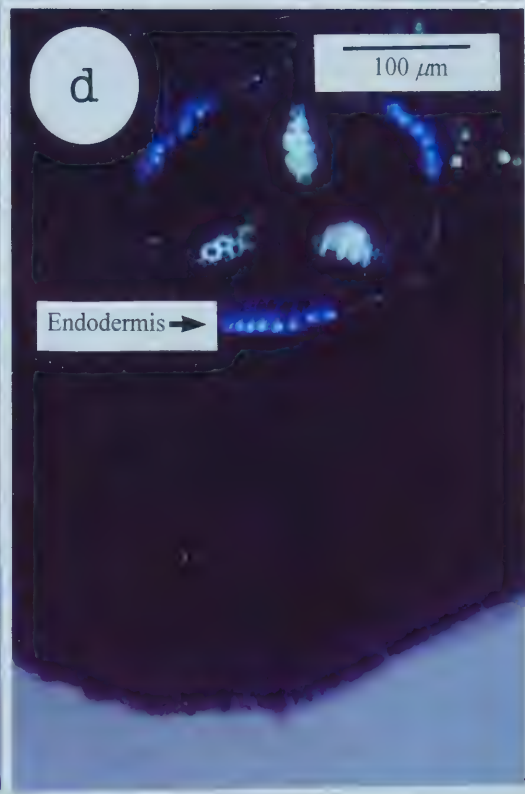
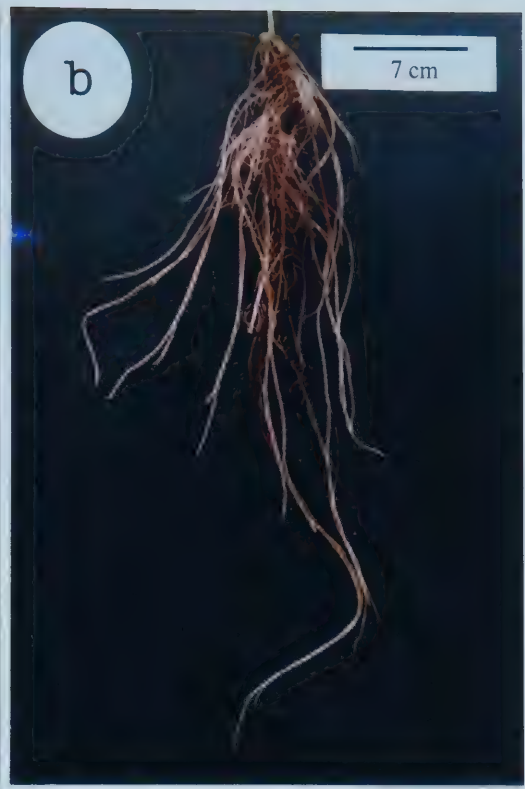


Figure 3.4. Relationship between water potential (ψ_w) and PTS₃ concentration in xylem exudate (A) and between root volume flow density (J_v) and PTS₃ concentration (B) for solution culture-grown aspen. Control (CTRL), mildly-stressed (M), and severely-stressed (S) treatments are shown. Linear regression is significant at $p \leq 0.05$ for (A) only.

Table 3.2. Stomatal conductance (g_s), water potential (ψ_w), root respiration, and root volume flow density (J_v) of control (CTRL) and stressed (S) aspen seedlings grown in solution culture. Means \pm SE (all $n=6$) are shown. Different letters indicate significant ($p \leq 0.05$) differences between treatments for each measurement.

Measurement	Treatment	
	Control (CTRL)	Stressed (S)
g_s (mmol m ⁻² s ⁻¹)	177.05 \pm 17.01 ^a	0.76 \pm 0.18 ^b
ψ_w (MPa)	-0.45 \pm 0.11 ^a	-2.00 \pm 0.18 ^b
Respiration (mg O ₂ L ⁻¹ m ⁻² s ⁻¹)	-0.085 \pm 0.018 ^a	-0.167 \pm 0.018 ^b
J_v (m ³ m ⁻² s ⁻¹ x 10 ⁻⁸)	3.26 \pm 0.62 ^a	1.22 \pm 0.65 ^b

Figure 3.5. Whole-root system morphology of trembling aspen seedlings grown in sand (4 months) (a) and solution culture (3.5 months) (b). Root cross-sections made at 30 mm from root tip from aspen roots grown in sand (4 months) (c) and solution culture (3.5 months) (d).



CHAPTER IV

Two Methods of Measuring Root Water Relations in Water-Stressed Trembling Aspen (*Populus tremuloides*) Seedlings

4.1 INTRODUCTION

Accurate measurements of root hydraulic conductivity (L_{pr}) are necessary for understanding the effects of physiological stresses on root water relations of woody plants. For root systems of small plants, the pressure chamber method of measuring L_{pr} has been used reliably (Radin and Eidenbock 1984, Boyer 1995, Henzler et al. 1999, Wan and Zwiazek 1999, Kamaluddin and Zwiazek 2001). However, measurement of L_{pr} requires that average volume flow density (J_v) be measured over time at a series of increasing pressures, which is a time-consuming process, and can contribute to the use of small sample sizes for a given treatment (Wan and Zwiazek 1999, Kamaluddin and Zwiazek 2001). Certain environmental stresses, such as nutrient deficiency (Radin and Eidenbock 1984), salinity (Cramer et al. 1986), and water deficit (Sperry et al. 1993, Wakamiya-Noborio et al. 1999), can result in variable physiological responses; a study of the physiological effects of drought stress might therefore require a large sample size due to response variability, but this is not always possible depending on the number and type of measurements taken per plant.

Drought stress is an important environmental stress that influences plant growth and physiology (Kozlowski 1985, Kozlowski and Pallardy 1997, Henzler et al. 1999, Steudle 2000). Studying the effects of drought stress on root water relations is problematic, not only because root systems are less accessible (Steudle 2000), but also

because the techniques used to measure water relations are destructive, or require that dehydrated roots be immersed in solution to measure L_{pr} . Such techniques may result in inaccurate water relations measurements of water deficit-stressed plants due to tissue damage or possibly due to tissue rehydration (Tyree et al 1995).

Recently, Tyree et al. (1995) developed a high-pressure flow meter (HPFM) capable of measuring root hydraulic conductance (K_r) of woody plants in situ, in the lab or the field. This method of measuring K_r is relatively quick and reliable, according to Tyree et al. (1995) and Tsuda and Tyree (2000). However, use of the HPFM to measure drought-stressed woody plants may result in an underestimation of K_r due to embolisms within xylem or rehydration of dehydrated tissues (Tyree et al. 1995, Dynamax 1996). If the HPFM will be used to measure K_r of stressed woody plants, as an alternative to more time-consuming, traditional measurement techniques, it is important to know the accuracy of measurements obtained using the HPFM.

The objectives of this study were to conduct and compare measurements of L_{pr} using the pressure chamber method and HPFM method in sand-grown and solution culture-grown aspen seedlings exposed to water deficit stress. The two measurements compared aspen subjected to different levels of water deficit stress with control seedlings. It was hypothesized that the pressure-chamber method would produce the most accurate measurements of L_{pr} .

4.2 MATERIALS AND METHODS

4.2.1 Sand-grown seedlings

4.2.1.1 Plant culture

In the first experiment, trembling aspen (*Populus tremuloides* Michx.) seedlings were used. Seedlings were grown from seed collected in Edmonton, Alberta, Canada. Seedlings were germinated in distilled water in Petri dishes. One-week-old germinants were planted into foam polymer-filled styroblock trays (Oasis potting medium, Beaver Plastics, Edmonton, AB) and grown for one month in a controlled environment growth chamber. The growth chamber conditions were as follows: 20°C (day)/ 20°C (night) temperature; 50% relative humidity (RH); and an 18-h photoperiod, with photosynthetically active radiation (PAR) of 275 $\mu\text{mol s}^{-1} \text{m}^{-2}$. Germinants were bottom-watered daily, and bottom-fertilized once a week with 0.3% 20-20-20 (N-P-K) fertilizer solution. Two-month-old seedlings were removed from the trays, their roots were cleaned of foam, and then planted two per pot into washed, non-sterile sand in 3-L plastic pots. Coarse sand was used to allow for a rapid induction of water deficit stress. The pots were lined with landscaping fabric to allow for drainage. Seedlings were grown for 2.5 months with the same watering schedule and growth chamber conditions as described above. Pots were randomly rotated once a week within the growth chamber to minimize the effects of the light intensity gradients within the chamber.

4.2.1.2 Water deficit stress treatments

Three water-deficit stress treatments were applied to 18-week-old seedlings: mild stress (MS), severe stress (SS), and severe stress recovery (SR). A control (CTRL) group consisted of seedlings that were watered with tap water daily. At 50% RH, mild stress was induced in 3 days to the point of slight wilting, and severe stress was induced in 4 days to the point of severe wilting in the leaves. Stress recovery was induced by rewatering severely-stressed seedlings for 24-h. Four seedlings were used in each stress treatment and control group.

4.2.1.3 Shoot water potential

Shoot water potential (ψ_w) for excised shoots was measured using a Scholander pressure chamber (PMS Instruments, Corvallis, OR) as previously described (Wan et al. 1999).

4.2.1.4 Root hydraulic conductivity measurements (high-pressure flow meter)

Root hydraulic conductivity was measured using a high-pressure flow meter (HPFM, Dynamax Inc., Houston, TX) (Tyree et al. 1995), while root systems remained in sand. Root flow rates were measured at steadily increasing pressures between 0 and 0.4 MPa. Root hydraulic conductance was calculated as the slope of the linear portion of the graph produced by the HPFM software, and was expressed against root surface area to calculate root hydraulic conductivity, $L_{pr(H)}$ ($\text{kg m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$).

4.2.1.5 Root hydraulic conductivity (pressure chambers)

Hydraulic conductivity was measured using Scholander pressure chambers (Wan and Zwiazek 1999). Root systems were immersed in distilled water at room temperature within the pressure chamber and subjected to a series of increasing hydrostatic pressures, from 0.25 to 1.0 MPa, at 0.05 MPa increments. Steady-state flow rate was allowed to equilibrate at each pressure, then measured for 20 minutes. Root flow rates were quantified by attaching a graduated glass pipette to the cut section of each root system using a short section of rubber tubing to measure changes in water volume over time. Volume change was averaged over the measurement time period at each pressure.

Root surface area was measured. Root systems were washed, surface dried, wrapped in aluminum foil, frozen in liquid nitrogen and broken into smaller fragments. The total surface area of the roots was measured with computerized scanning (Sigma Scan 4.0, Jandel Scientific, San Rafael, CA). Root volume density, J_v ($\text{m}^3 \text{m}^{-2} \text{s}^{-1}$) was calculated by expressing root flow rate over root surface area. Root hydraulic conductivity, L_{pr} ($\text{m}^3 \text{m}^{-2} \text{s}^{-1} \text{MPa}^{-1}$) was calculated as the slope of the regression line of J_v plotted against hydrostatic pressure.

This experiment was repeated twice with sand-grown seedlings, with measurements of L_{pr} taken for each seedling, but only one of these experiments also included $L_{pr(H)}$ measurements. Results from the repeated experiments are not shown in this study.

4.2.2 Solution culture-grown seedlings

4.2.2.1 Plant culture

In the second experiment, trembling aspen seedlings were used. Seedlings were germinated and placed into styroblock trays, as described earlier for sand-grown aspen. Germinants were grown for one month in a controlled environment growth chamber with the following conditions: temperature, 22°C (day)/ 18°C (night); 60% RH; and a 16-h photoperiod, with 285 $\mu\text{mol s}^{-1} \text{m}^{-2}$ of PAR at plant height. The same watering schedule was used as for sand-grown seedlings. Five week-old seedlings were removed from the styroblocks, roots were cleaned of foam, and placed into aerated solution culture with roots immersed in 25% Hoagland's solution. The solution was increased to 50% Hoagland's solution after one week, and then to full-strength Hoagland's solution after another three days. The mean dissolved O₂ concentration was $7.49 \pm 0.21 \text{ mg L}^{-1}$. Seedlings were grown in solution culture in the same growth chamber for one month, with the solution culture changed each week.

4.2.2.2 Water deficit stress treatments

Two-month-old seedlings were subjected to two water deficit stress treatments: mild stress (M), and severe stress (S). Two stress levels were used to determine if there is a difference in the response of aspen to different levels of water deficit. Both water stress treatments were induced by placing roots of intact seedlings into a sealed, aerated, high-humidity (85-90% RH) container within the growth chamber. Mildly-stressed seedlings exhibited slight leaf wilting in approximately 19 hours, and severely-stressed

seedlings exhibited severe leaf wilting after approximately 22 hours. A control (CTRL) group consisted of seedlings in aerated solution culture. Eight seedlings were used in each stress treatment and control group.

4.2.2.3 Shoot water potential and root hydraulic conductivity

Water potentials (ψ_w) of excised shoots were measured, and L_{pr} measurements were conducted with pressure chambers and an HPFM as described above. For HPFM measurements, control seedlings remained in solution culture, while stressed seedlings remained in the high-humidity chamber in which they were stressed.

This experiment was previously conducted three times using sand-grown aspen seedlings. In all three experiments, L_{pr} measurements were taken, but $L_{pr(H)}$ measurements were only taken in two of those experiments.

4.2.3 Statistical analysis

For both experiments, differences in mean ψ_w , mean L_{pr} , and mean $L_{pr(H)}$ between the stress treatment and control groups were analyzed for statistical significance by univariate ANOVA (SPSS 10.0, SPSS Inc., Chicago, IL). Relationships between ψ_w , L_{pr} , and $L_{pr(H)}$, were analyzed for significance using linear regression analyses ($y = mx + b$) for both experiments. For all tests, the critical p-value was set at 0.05.

The statistical model used for univariate ANOVA using a completely randomized design was: $Y_{ij} = \mu + t_i + e_{ij}$

where Y_{ij} = value of individual observation (i = treatment, j = observation)

μ = overall mean

t_i = effect of i^{th} treatment (where i = level of water deficit stress)

e_{ij} = random error

4.3 RESULTS

4.3.1 Root water relations in sand-grown seedlings

Shoot water potential (ψ_w) decreased in water deficit-stressed sand-grown aspen seedlings (Fig. 4.1A). ψ_w decreased from approximately -0.75 MPa in control seedlings to -1.3 in mildly-stressed (MS) seedlings. ψ_w of severely-stressed (SS) seedlings was significantly ($p \leq 0.05$) lower than for control seedlings, and ψ_w in stress-recovered (SR) aspen showed a significant ($p \leq 0.05$) increase compared to severely-stressed seedlings (Fig. 4.1A). In repeated experiments, SS seedlings had water potentials from -2 to -4 MPa, with some recovery of ψ_w seen in SR seedlings (data not shown).

The regression slope (not shown) of the relationship between J_v and pressure from 0.25 to 0.55 MPa were used to calculate L_{pr} values for each seedling. Root hydraulic conductivity measured with pressure chambers (L_{pr}) increased with increasing water deficit stress in sand-grown aspen seedlings (Fig. 4.1B). L_{pr} in MS seedlings were similar to control seedlings (Fig. 4.1B). L_{pr} increased almost two-fold in SS seedlings, and decreased over seven-fold in SR seedlings, compared to controls. Similar means were observed when L_{pr} was calculated at higher pressures from 0.65 to 1.0 MPa (data not shown).

Root hydraulic conductivity measured with a high-pressure flow meter ($L_{pr(H)}$) showed non-significant increases with increasing water deficit stress (Fig. 4.1C). Mean

$L_{pr(H)}$ for MS and SS seedlings increased three-fold and four-fold, respectively, compared to control seedlings (Fig.4.1C). The mean $L_{pr(H)}$ for SR seedlings returned to control levels after 24 h of rewatering (Fig. 4.1C). Differences in $L_{pr(H)}$ values for all treatment and control groups were not significant.

L_{pr} and $L_{pr(H)}$ were positively correlated with ψ_w , but the correlation was only significant ($p \leq 0.05$) for L_{pr} (Fig.4.2A, B). Repeated experiments have also shown a positive correlation between L_{pr} and ψ_w , with a lack of a significant correlation between $L_{pr(H)}$ and ψ_w (data not shown). There was also a significant positive correlation between L_{pr} and $L_{pr(H)}$ ($p \leq 0.05$) (Fig. 4.2C). The same correlation between L_{pr} and $L_{pr(H)}$ was observed in a repeated experiment.

4.3.2 Root water relations in solution culture-grown seedlings

Shoot water potential (ψ_w) decreased with increasing water deficit stress in solution culture-grown aspen seedlings (Fig. 4.3A). ψ_w decreased from approximately -0.7 MPa in control seedlings to -2.3 MPa in severely-stressed (S) seedlings, with no significant change in ψ_w of mildly-stressed (M) seedlings relative to controls (Fig.4.3A). In repeated experiments, S seedlings had a slightly wider range of water potentials than SS treatments of sand-grown aspen, from -1.5 to -3 MPa (data not shown).

The regression slope (not shown) of the relationship between J_v and pressure from 0.25 to 0.95 MPa were used to calculate L_{pr} values. L_{pr} for the M treatment was approximately twice that of control seedlings (Fig. 4.3B). L_{pr} for the S treatment was approximately half that of control seedlings (Fig. 4.3B).

$L_{pr(H)}$ showed non-significant increases with water deficit stress compared to controls (Fig. 4.3C). $L_{pr(H)}$ for M seedlings increased five-fold, compared to control seedlings, and S seedlings had an $L_{pr(H)}$ approximately twice that of control seedlings (Fig. 4.3C).

L_{pr} was positively correlated with ψ_w , but was not significant (Fig. 4.4A). Correlations between $L_{pr(H)}$ and ψ_w (Fig. 2.4B) and between $L_{pr(H)}$ and L_{pr} did not show any trend and were not significant (Fig. 4.4C).

4.4 DISCUSSION

4.4.1 *Water relations and root hydraulic conductivity (pressure chamber method)*

The pressure chamber is a more conventional method of measuring L_{pr} and has been used in several experiments (Radin and Eidenbock 1984, Hanson et al. 1985, Moon et al. 1986, Henzler et al. 1999, Wan and Zwiazek 1999, 2001). The advantages of this method are that: 1) water flow is in the same direction as that of the transpirational flow, from roots to shoot; 2) and that solutes, such as dissolved minerals and ions present in the water or the root, are flushed from the root, preventing the interference of osmotic potential with measurements of L_{pr} (Tyree et al. 1995). Water that enters the root will, therefore, encounter areas of resistance to water flow, such as the endodermis and exodermis. Under stress, xylem embolisms, which would ordinarily lower or possibly cease root water flux (Tyree and Sperry 1988, 1989), can be flushed out partly or entirely from the root xylem (Sperry et al. 1988). Therefore, L_{pr} measurements of water deficit-stressed plants may be largely dependent on the resistance of dehydrated tissues to water flux, with greater pressures required to induce water flux in more severely-stressed plants

(Boyer 1995). One disadvantage of pressure chambers is that flooding of intercellular spaces may occur, thereby altering typical pathways of water flow (Skinner and Radin 1994). Intercellular flooding has resulted in an overestimation of the percentage of water flux through the apoplastic pathway of *Glycine max* (Steudle and Boyer 1985) and *Vigna radiata* (Salim and Pitman 1984), which may subsequently result in an overestimation of L_{pr} . It is not known if pressure chamber-based measurements of L_{pr} actually do overestimate the actual L_{pr} .

In the present study, ψ_w decreased with water deficit stress in both sand-grown and solution culture-grown aspen seedlings, although ψ_w values were slightly lower in sand-grown seedlings (Figs. 4.1A, 4.3A). The higher control ψ_w values in solution culture-grown aspen (data not shown) may be due to the fact that water is abundant in solution culture, unlike sand which has a low water-holding capacity. Controls for both sand-grown and solution culture-grown aspen were higher than -1 MPa, and are similar to ψ_w values obtained from other studies (Wan et al. 1999, Wan and Zwiazek 1999).

Water deficit resulted in a reduction in root hydraulic conductivity measured by the pressure chamber method (L_{pr}) in solution culture-grown seedlings (Fig. 4.3B), but not in sand-grown seedlings (Fig. 4.1B). However, the significant positive correlation between ψ_w and L_{pr} (Fig. 4.2A) for sand-grown aspen suggests that L_{pr} decreased with decreasing ψ_w , which is an indicator of water deficit stress. Repeated experiments with sand-grown aspen (Chapter II) showed that L_{pr} decreased with increasing water deficit stress.

The non-significant correlation between ψ_w and L_{pr} in solution culture-grown seedlings (Fig. 4.4A) is likely due to the more rapid water deficit to which these seedlings

were subjected. Rapid and gradual water deficits cause different drought resistance mechanisms to be utilized by seedlings, and can therefore change the relationships between the different water relations parameters that were measured. Furthermore, rapid water deficit stress may affect measurements of stomatal conductance (g_s) and L_{pr} more quickly due to rapid localized dehydration of shoot and root tissues, respectively. Gradual water deficit stress could allow all water relations parameters to decrease concurrently because changes in L_{pr} are affected by changes in ψ_w and g_s (Tsuda and Tyree 2000), and so the response to water deficit stress would be at a whole-plant level. Because of the differences in the time required to induce water deficit stress in aspen grown in solution culture versus sand, results from sand- and solution culture-grown aspen are not directly comparable.

The difference in the effects of water deficit treatments on L_{pr} values illustrates the difficulty in replicating repeated experiments. Applying consistent levels of mild and severe water deficit stress to sand-grown aspen seedlings was more difficult because of the variability in the responses of individual seedlings to gradual water deficit stress, whereas solution culture-grown seedlings were stressed more uniformly and more quickly. As a result, although seedlings within a treatment or control group were treated as uniformly as possible in this study and for each repeated experiment, uniform treatment did not guarantee a similar response to water deficit stress. Because water deficit and embolism-producing experiments have shown that water deficit stress can produce a variable response (Sperry et al. 1993, Wakamiya-Noborio et al. 1999), it is possible that sand-grown severely-stressed seedlings were more similar to mildly-stressed seedlings, and that sand-grown stress-recovered seedlings were more similar to severely-

stressed seedlings in their stress response (Fig. 4.1B). Repeated experiments have also shown that stress-recovered seedlings sometimes but do not always recover from severe stress within 24 h of rewatering (data not shown).

It was expected that ψ_w and L_{pr} would decrease with increasing water deficit stress. Previous experiments (Chapters II, III, in this thesis) have shown that water potential (ψ_w) decreased with water deficit stress (Pierce and Raschke 1980, Mott and Parkhurst 1991, Schulze 1993), as did root hydraulic conductance (K_r) and root hydraulic conductivity (L_{pr}) (Cruz et al. 1992, North and Nobel 1992, 1996, Dubrovsky et al. 1998, Lo Gullo et al. 1998, Lu and Neumann 1999). Reduction of L_{pr} with water deficit stress in trembling aspen seedlings was also observed (Chapters II and III in this study). Some recovery of L_{pr} following water deficit stress has also been observed (North and Nobel 1992, 1996, Lo Gullo et al. 1998). High xylem tensions that occur during water deficit can result in embolism formation, which can reduce L_{pr} (Tyree and Sperry 1988, 1989, Nardini and Pitt 1999). Root tissues such as the endodermis and exodermis can also become thickened and suberized in response to water deficit, which can also lower root water flux (Cruz et al. 1992, North and Nobel 1996, Lo Gullo et al. 1998), and also delay stress recovery when water becomes available because of the greater resistance to water flux.

4.4.2 Water relations and root hydraulic conductivity (HPFM method)

Root water flux measured with a high-pressure flow meter (HPFM) has been shown to produce similar K_r values compared to the evaporative flux method (Tsuda and Tyree 1997, 2000) and the pressure chamber method (Tyree et al. 1995). However, it has

been noted that there are limitations to this method. Embolisms within the root xylem may produce a non-linear relationship between root flow, F (kg s^{-1}) and pressure using the HPFM due to air bubble compression (Tyree et al. 1995, Dynamax 1996). Tissue rehydration may also result in an underestimation of K_r during measurements (Tyree et al. 1995, Dynamax 1996, Tsuda and Tyree 2000). The direction of water flow, from the cut stem into the root, is also in the opposite direction to the transpirational flow of water (Tyree et al. 1995), and opposite to the flow of water through roots when root systems are pressurized in the pressure chamber. Water entering the cut stem does not have to move through potential areas of resistance to water flow such as the endodermis and exodermis in order to enter the xylem, and therefore bypasses these areas of resistance. Dissolved solutes, such as minerals and ions which could be present in the root system itself would, therefore, accumulate in roots instead of being flushed out of roots, and the resulting changes in osmotic potential could affect measurements of root water flux (Tyree et al. 1995). The fact that HPFM calculates K_r by measuring the pressure differences between the root and the pressurized water (Dynamax 1996), instead of directly measuring flow rate, means that values for L_{pr} and root hydraulic conductivity measured with the HPFM ($L_{pr(H)}$) will be in a different range and, therefore, not directly comparable. L_{pr} values obtained using pressure chambers may be closer than $L_{pr(H)}$ values to the true conductance values (Tyree et al. 1995) because the HPFM has different sources of error (problems resulting from direction of water flow, method of K_r calculation, and use of low hydrostatic pressure), which were previously mentioned in this section. The pressure chamber does not have these sources of error. One advantage of using pressure chambers for water deficit-stressed plants is that when embolisms are present, a better measure of

hydraulic conductivity can be obtained using greater hydrostatic pressures to remove some of the embolisms (Tyree et al. 1995), while it is not advised to use pressures greater than 5.0 MPa with the HPFM (Dynamax 1996). Dehydrated root systems may not produce linear L_{pr} measurements at low pressures. However, the main interest in comparing the two methods of measuring root hydraulic conductivity was to determine if there was a significant correlation between both methods.

The HPFM was able to detect changes in the $L_{pr(H)}$ of sand- and solution-culture grown seedlings. Both sand-grown and solution culture-grown aspen showed generally similar trends in response to water deficit stress, compared with L_{pr} , despite differences in the magnitude of the values and the standard errors. $L_{pr(H)}$ values of sand-grown seedlings increased with increasing water deficit, and were lower in stress-recovered seedlings compared to controls, although differences were not significant (Fig. 4.1C). Similarly, L_{pr} values for these seedlings increased with increasing water deficit (Fig. 4.1B). For solution culture-grown aspen, $L_{pr(H)}$ increased in mildly-stressed seedlings compared to controls, and were slightly higher in severely-stressed seedlings than in controls (Fig. 4.3C). However, differences in $L_{pr(H)}$ were not significantly different. This differed slightly from L_{pr} values for solution culture-grown aspen, which increased in mildly-stressed seedlings, and then decreased in severely-stressed seedlings compared to controls (Fig. 4.3B). Although the HPFM was able to detect differences in $L_{pr(H)}$ values to the same extent as the pressure chamber, the standard errors were considerably larger for $L_{pr(H)}$ values, resulting in no significant differences. Furthermore, the HPFM indicated that the $L_{pr(H)}$ of solution culture-grown severely-stressed seedlings was twice as high than that of control seedlings, when the pressure chamber indicated that the L_{pr} of

severely-stressed seedlings was half that of control seedlings. Because of the discrepancy between $L_{pr(H)}$ and L_{pr} measurements, there was no relationship between the two parameters (Fig. 4.4C) or between $L_{pr(H)}$ and ψ_w (Fig. 4.4B).

Compared to $L_{pr(H)}$ values in previous studies (Tsuda and Tyree 2000), the values from the present study are considerably smaller because previous studies used field-grown plants and woody plants with much larger root systems, which would likely result in greater $L_{pr(H)}$ values.

Because the HPFM produces variable measurements of $L_{pr(H)}$, it may not be as reliable as the pressure chamber for measurements of root hydraulic conductivity in water deficit-stressed seedlings. Although the relationship between $L_{pr(H)}$ and L_{pr} for sand-grown aspen was significant (Fig. 4.2C), a repeated experiment with sand-grown aspen showed a similar relationship but was not statistically significant (data not shown). For solution culture-grown aspen, there was no relationship between $L_{pr(H)}$ and L_{pr} (Fig. 4.4C). The fact that one experiment with sand-grown aspen showed a significant relationship, when a repeated experiment and an experiment involving solution culture-grown aspen did not show significance, suggests that the HPFM produces variable measurements that do not always coincide with pressure chamber-based measurements.

If the HPFM is to be used for $L_{pr(H)}$ measurements of water deficit-stressed plants, then its measurements should show a significant relationship with a parameter that is being used as an indicator of water deficit stress, such as ψ_w . Other experiments with aspen have shown that ψ_w is a good indicator of water deficit stress (Chapters II and III). This study showed that the relationships between $L_{pr(H)}$ measurements of both sand- and solution culture-grown aspen and ψ_w were not significant (Figs. 4.2B, 4.4B). The lack of

significance between ψ_w and $L_{pr(H)}$, particularly for solution culture-grown aspen where there was no relationship, suggests that the HPFM is not sufficiently sensitive for measuring changes in $L_{pr(H)}$ for water deficit-stressed seedlings. This may be due to tissue rehydration within the root, or bubble compression of any embolisms present within the root, both of which could result in inaccurate measurements of $L_{pr(H)}$.

The potential sources of error stated in the HPFM manual (Dynamax 1996) which are discussed above in this section suggest that the HPFM is not suitable for use with water deficit-stressed plants. This experiment confirms that HPFM measurements are quite variable. While this may be partly due to the method in which $L_{pr(H)}$ is measured by the HPFM, the lack of significance in the relationship between $L_{pr(H)}$ and L_{pr} , or between $L_{pr(H)}$ and ψ_w , may also be partly related to small sample size. Because of the time-consuming nature of the L_{pr} measurements made with the pressure chamber, small sample sizes were used. However, because the HPFM offers a quick method of measuring, a larger sample size may reduce some of the variability observed in the present study. Further experimentation with the HPFM using larger sample sizes would be needed to determine if the HPFM could be a useful instrument for measuring $L_{pr(H)}$ of seedlings subjected to water deficit stress.

In conclusion, ψ_w and root hydraulic conductivity (L_{pr} , $L_{pr(H)}$) decreased with water deficit stress in aspen grown in both sand or solution culture. Both the HPFM and the pressure chamber were able to measure changes in root hydraulic conductivity due to water deficit stress. Both the HPFM and pressure chamber methods have limitations that could result in an underestimation or overestimation of L_{pr} , respectively. A correlation

between ψ_w , and L_{pr} indicated that the pressure chamber consistently detected water deficit-induced changes in L_{pr} . Because parameters such as ψ_w and g_s are used to estimate the extent of water deficit stress in experiments prior to harvesting, it is useful for L_{pr} measurements to be correlated with such estimates of water deficit stress. The variability of $L_{pr(H)}$ measurements with the HPFM resulted in no significant differences in $L_{pr(H)}$ measurements between treatment and control groups, a lack of a significant relationship between ψ_w and $L_{pr(H)}$, and a relationship between $L_{pr(H)}$ and L_{pr} that was only significant in one experiment. This study indicates that the HPFM is not sufficiently sensitive to detect changes in $L_{pr(H)}$, and therefore may not be a reliable means of consistently measuring $L_{pr(H)}$. The variability associated with the HPFM may be partly due to the usage of small sample sizes, such as those used in the present study. Based on the present study, the pressure chamber method appears to be able to detect differences in L_{pr} of water-stressed seedlings in experiments where smaller sample sizes are used, based on the significant differences between treatment and control groups, and because of the significant relationship between L_{pr} and ψ_w . However, further experimentation is necessary to determine if the variability produced by HPFM measurements could be reduced by larger sample sizes. The HPFM may therefore be useful only for experiments in which large sample sizes are to be used because of the variability in $L_{pr(H)}$ measurements. Although the HPFM may provide a quick measure of $L_{pr(H)}$, it is important that such tests regarding the reliability of the HPFM be conducted in order to determine if the HPFM is suitable for all types of plants subjected to the required experimental conditions.

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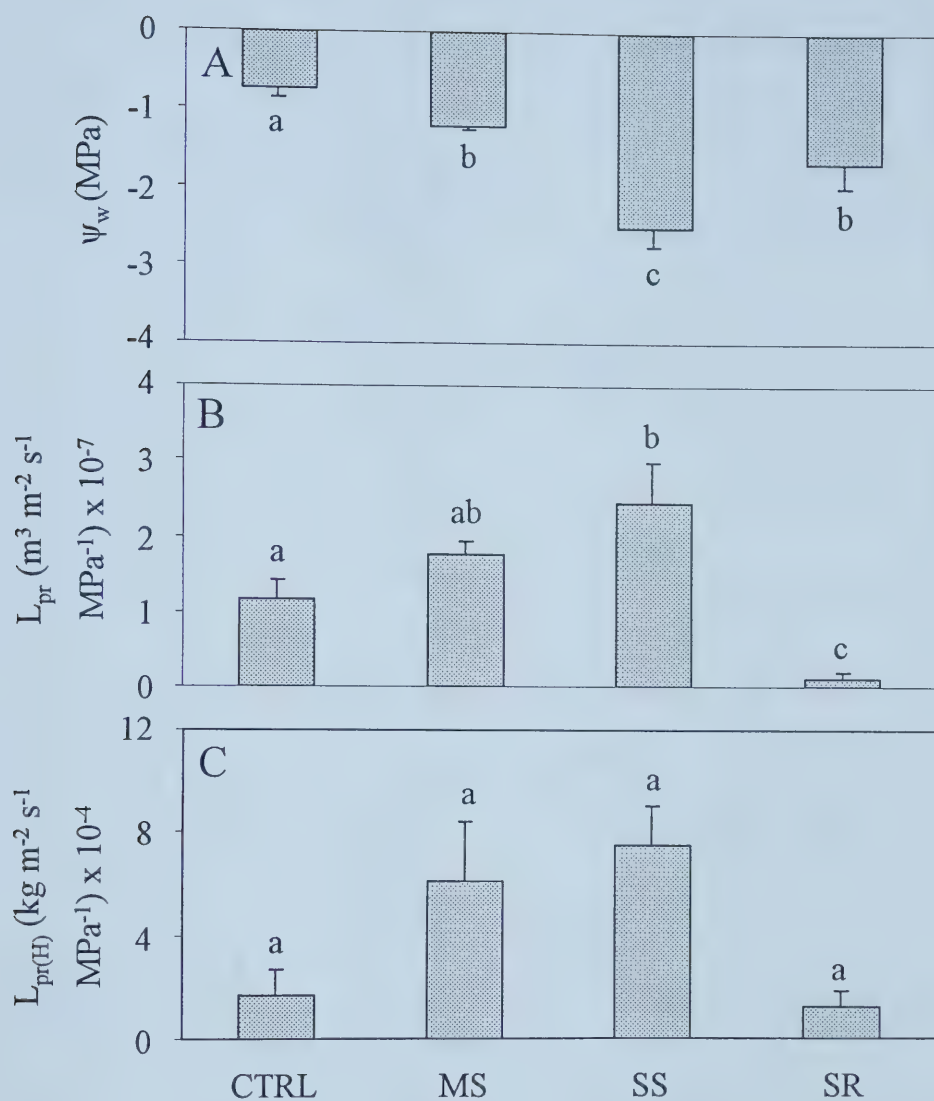


Figure 4.1. Water potential (ψ_w) (A), and root hydraulic conductivity measured with pressure chambers (L_{pr}) (B) and high-pressure flow meter ($L_{pr(H)}$) (C), for control (CTRL), mildly-stressed (MS), severely-stressed (SS), and stress-recovered (SR) aspen seedlings. Aspen were grown in sand. Bars with different letters are significantly different ($p \leq 0.05$). Means + SE are shown (all $n=4$).

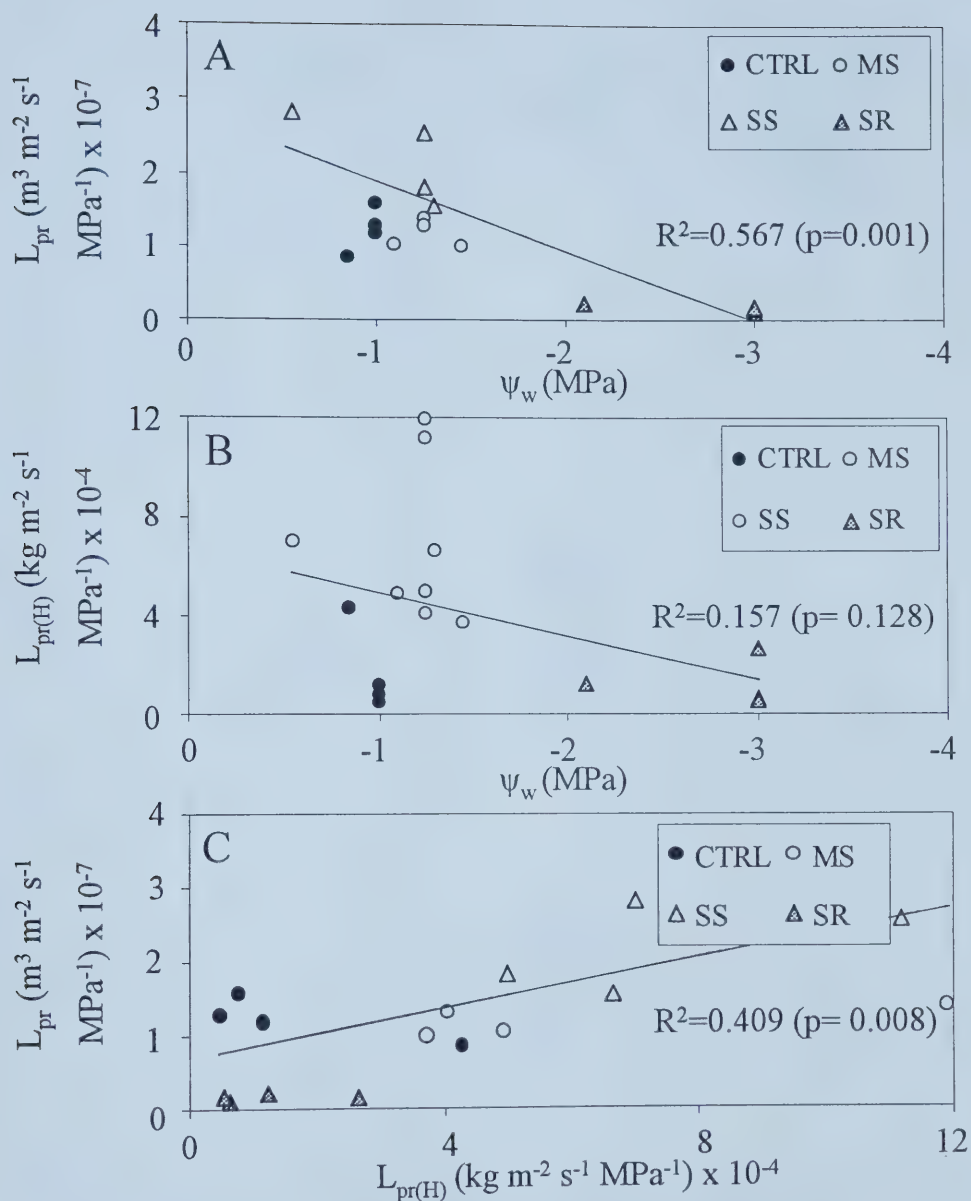


Figure 4.2. Relationship between water potential (ψ_w) and root hydraulic conductivity measured with pressure chambers (L_{pr}) (A) and a high-pressure flow meter ($L_{pr(H)}$) (B); and between $L_{pr(H)}$ and L_{pr} (C). Control (CTRL), mildly-stressed (MS), severely-stressed (SS), and stress-recovered (SR) treatments are shown. Aspen were grown in sand. Linear regressions for (A) and (C) were significant at $p \leq 0.05$.

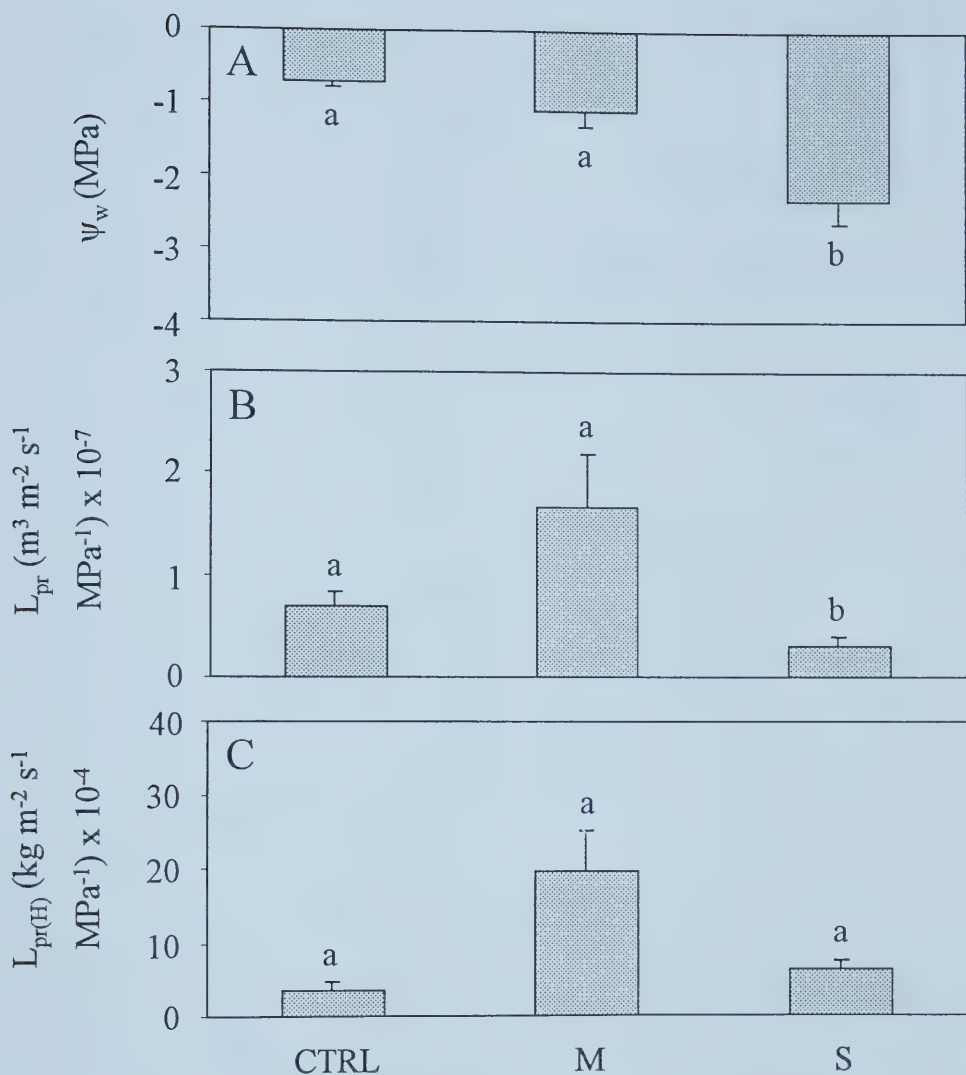


Figure 4.3. Water potential (ψ_w) (A), and root hydraulic conductivity of control (CTRL), mildly-stressed (M), and severely-stressed (S) aspen measured with pressure chambers (L_{pr}) (B) and a high-pressure flow meter ($L_{pr(H)}$) (C). Aspen were grown in solution culture. Bars with different letters are significantly different ($p \leq 0.05$). Means + SE are shown (all $n=8$).

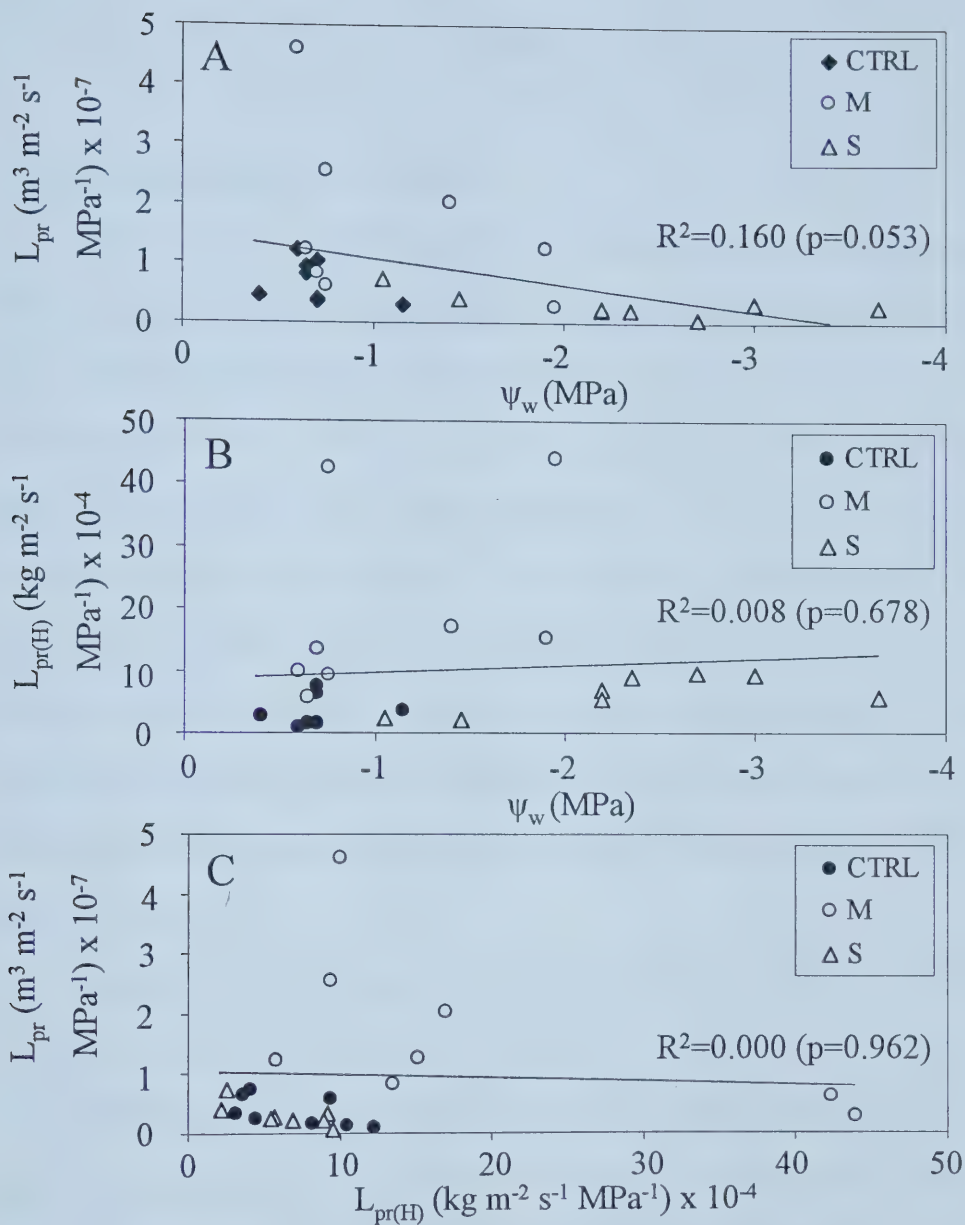


Figure 4.4. Relationship between water potential (ψ_w) and root hydraulic conductivity measured with pressure chambers (L_{pr}) (A) and a high-pressure flow meter ($L_{pr(H)}$) (B); and between $L_{pr(H)}$ and L_{pr} (C). Aspen were grown in solution culture. Control (CTRL), mildly-stressed (M), and severely-stressed (S) treatments are shown. Linear regressions were not significant at $p \leq 0.05$.

CHAPTER V

Synthesis

5.1 General Discussion and Conclusions

The overall objective of these studies was to add to the current knowledge about effects of water deficit stress and rehydration on the root water relations of trembling aspen seedlings grown in sand and in solution culture. The focus of Chapter II was to show the effects of water deficit stress on root water relations of sand-grown seedlings. Chapter III focused on the effects of water deficit stress on the root water relations of solution culture-grown aspen seedlings. Finally, Chapter IV compared two methods of measuring root hydraulic conductivity of water deficit-stressed seedlings. Aspen seedlings were grown in both sand and solution culture to produce differences in root morphology and anatomy that had the potential to affect root water relations during water deficit stress. Sand was used as the solid growth medium because of its low water-retention properties.

Stomatal conductance (g_s), shoot water potential (ψ_w), root volume flow density (J_v), and root hydraulic conductivity (L_{pr}) were observed to decrease with increasing water deficit stress in sand- and solution culture-grown seedlings (Chapter II, III), as reported in previous studies (Cruz et al. 1992, Tyree et al. 1992, Sperry et al. 1993, Lo Gullo et al. 1998, Lu and Neumann 1999, Nardini and Pitt 1999, Sparks and Black 1999). Values of g_s , ψ_w , and L_{pr} in the present study were similar to those of previous studies (Wan and Zwiazek 1999, Wan et al. 1999, Kamaluddin and Zwiazek 2001), with some differences due to the age and size of seedlings. The positive correlations between these

parameters indicate that response to water deficit affects the entire plant, as these parameters are interdependent.

AQPs may be able to metabolically regulate root water flow during water deficits to prevent water loss from roots. The present study showed that AQPs were not as affected by mercuric inhibition during severe water stress compared to AQPs in control and mildly-stressed seedlings, indicating that AQPs were already largely deactivated by severe water deficit (Chapter II, III). Reduction in mercuric inhibition of aquaporins (AQPs) with increasing water deficit stress (Martre et al. 2001) and in metabolically less active AQPs (Zhang and Tyerman 1999) has been previously reported. Water stress-induced deactivation of AQPs in mildly-stressed aspen resulted in an increase in activation energy (E_a) with water deficit stress in the present study (Chapter II), because of the increased resistance to water flow through cell membranes. The increase in E_a further suggests that AQPs were deactivated. AQP deactivation resulted in increases in the percentage of apoplastic flow through roots, suggesting that AQPs are responsible for a substantial amount of root water flow, and that apoplastic flow is more important during severe stress and stress recovery. AQPs may have an important regulatory role in cell-to-cell root water flow, as AQP blockage resulted in significant reductions in J_v , while increasing the proportion of apoplastic root water flow (Chapter II, III). Apoplastic flow calculated from the present study was much higher than in previous studies (Hanson et al. 1985, Moon et al. 1986, Skinner and Radin 1994, Wan and Zwiazek 1999, Kamaluddin and Zwiazek 2001). There are several problems with using apoplastic dyes, which cannot be used to accurately quantify apoplastic flow (Moon et al. 1986, Skinner and Radin 1994, Steudle and Peterson 1998). However, changes in dye concentration

may be useful for interpretation of stress-induced changes in root water relations.

Although mercaptoethanol has been used to partly and fully reverse mercuric inhibition (Tyerman et al. 1999, Wan and Zwiazek 1999, Kamaluddin and Zwiazek 2001), it is a metabolic poison. The concentration of ME was likely too high to observe reversal of mercuric inhibition, as mentioned in Chapters II and III.

The morphological and anatomical differences between sand- and solution culture-grown seedlings likely contributed to the increased resistance of sand-grown seedlings to moderate water deficit stress (Chapter II, III). The lack of an exodermis in solution culture-grown plants has been previously reported (Freundl et al. 1998, Zimmerman and Steudle 1998, Wan and Zwiazek 2001). The presence of an exodermis and suberized tissue formed during water deficit stress increases resistance to water flow, and can hinder stress recovery after rewatering (Cruz et al. 1992, North and Nobel 1996, Lo Gullo et al. 1998), which may partly explain the lack of recovery in sand-grown aspen in the present study (Chapters II and IV). The exodermis was also partly responsible for the reduction in permeability of roots to HgCl_2 in sand-grown aspen compared to solution culture-grown aspen (Chapters II and III). The larger L_{pr} values of sand-grown seedlings were likely due to increased root surface area compared to solution culture-grown seedlings.

Root respiration increased with water deficit stress in sand- and solution culture-grown aspen (Chapter II, III). Because higher respiration rates denote increased metabolic activity, it is possible that the increase in respiration was due to changes in AQP activity as a result of metabolic AQP regulation, but further experiments would be needed to address the relationship between respiration rate and AQP activity. It is also

possible that the addition of water during respiration measurements resulted in localized and immediate rehydration effects, more so in stressed seedlings than in control seedlings, without having an immediate effect on J_v .

Although both the pressure chamber and the high pressure flow meter (HPFM) were able to detect changes in root hydraulic conductivity (L_{pr} , $L_{pr(H)}$), the HPFM was not sufficiently sensitive in its detection of differences in $L_{pr(H)}$, particularly in solution culture-grown seedlings. This was indicated by the non-significant relationship between $L_{pr(H)}$ and ψ_w for aspen grown in sand and in solution culture, the lack of a relationship between $L_{pr(H)}$ and L_{pr} , and the lack of significant differences in $L_{pr(H)}$ between treatments (Chapter IV). Some of the variability may be reduced by the use of larger sample sizes, but further experiments are needed to confirm if increased samples would reduce some of the variability. Because of the potential sources of error inherent in HPFM measurements, the HPFM may not be suitable for measurements involving water deficit-stressed plants. The pressure chamber (Skinner and Radin 1994, Boyer 1995) and the HPFM (Tyree et al. 1995, Tsuda and Tyree 2000) both have different sources of error associated with measurements, but it was not possible to determine which method resulted in a larger deviation from the actual L_{pr} of the root systems. However in this study, the pressure chamber seemed to produce more consistent measurements of L_{pr} for water deficit-stressed seedlings, given the small sample sizes used.

5.2 Technical Research Problems and Potential Solutions for Future Experiments

5.2.1 *Seedlings*

Several technical problems were encountered in this study relating to the experimental procedures, which could likely have affected the results of this study to some degree. The method of inducing water deficit stress used in these experiments is a fairly rapid process, compared to drought stress that might occur in the field. Therefore, the results of this study may not reflect drought stress responses occurring in the field, or those induced over a longer time in the lab. This is because aspen may use different mechanisms to deal with short-term versus longer-term water deficit (discussed in Chapters II and III). Withholding water from sand-grown aspen did not produce a uniform stress response within each treatment group for several of the measured parameters (Chapter II), although all aspen seedlings were treated as uniformly as possible prior to and during the experiments. Although this was particularly true for sand-grown aspen, a variable response was also observed with solution culture-grown aspen. Additionally, it was difficult to induce the same levels of water deficit stress, such as mild stress and severe stress, in each repeated experiment, which resulted in data that varied between experiments. Therefore the results shown in Chapters II-IV are results which are representative of those observed from the majority of the repeated experiments conducted. Because trembling aspen is a tree species that grows relatively quickly after approximately 1.5 months of age, it was also difficult to maintain the same age of seedlings in all experiments. A difference of a couple of weeks in the age of the

seedlings could have produced some variability between the experiments, but not likely within the experiments.

The use of small sample sizes resulted in the necessity to repeat experiments in order to consistently observe the effects of water deficit stress. Because a number of the measurements conducted, such as root hydraulic conductivity (L_{pr}) and activation energy (E_a) were time-consuming it was not possible to have a larger sample size. Furthermore, a single experiment required a few days for completion. Variability in the response to water deficit stress within a treatment group was likely greater due to small sample size. As a result of some of these technical difficulties, the response of aspen seedlings to water deficit stress may not have been statistically significant, but the same trend may have been observed over several experiments. Therefore, a lack of significance did not necessarily indicate that stress had no effect, but may have indicated that the effect was not statistically detectable due to variability in seedling response.

A second problem with seedlings in this study was that sand-grown aspen were used for most of the study prior to knowing how their root anatomy differed from solution culture-grown aspen which had been used previously in the lab for all the types of experiments conducted in this study. It was only discovered late in this study that sand-grown aspen seedlings possess an exodermis, but solution culture-grown aspen do not (Chapter III). It would seem that, based on several repeated experiments where $HgCl_2$ did not affect AQPs of sand-grown aspen at 0.3 MPa (data not shown), that higher pressures (1.0 MPa) were required for sand-grown aspen. Because root anatomy was not known, no experiments were conducted to test the permeability or function of the exodermis.

Because water deficit stress can produce a variable response in plants, one cannot minimize this experimental difficulty if all seedlings are being treated uniformly and if experiments are conducted consistently. Using a completely randomized block design, where time is the blocking factor may reduce some variability, but may not be effective in reducing the observed variability in this study. Equipment such as the HPFM may allow for more rapid measurements of root hydraulic conductivity, although there are several problems in using the HPFM with water deficit-stressed seedlings (Chapter IV). However, a large sample size could be used if the HPFM is to be used, and this may reduce some of the variability in root hydraulic conductivity seen in aspen seedlings, as well as some of the variability associated with measurements from the HPFM.

Before a new plant species, or a similar species grown under different conditions is used for root water relations experiments, root anatomy and morphology should be studied prior to starting experiments. These differences may help to interpret experimental results and to design experiments that take anatomical and morphological traits into account.

5.2.2 Equipment

Some of the experiments were conducted before renovations to the principal growth chamber used in these experiments were completed, whereas other experiments were conducted following renovations. Based on results of experiments prior to and following renovations, it would seem that growth chamber conditions changed, thereby changing stress response. The three main changes were that: 1) relative humidity (RH) greatly increased from approximately 45% to 60%; 2) fluorescent and incandescent bulbs

were replaced by full-spectrum fluorescent light bulbs, which did no change the quantum but may have changed the proportion of red-spectrum to blue-spectrum light available to seedlings; and 3) the ventilation system was changed. As a result of these changes, stomatal conductance (g_s) increased nearly ten-fold following renovations, and it took nearly four times longer to induce water deficit stress as a result of the increase in RH (Chapter II, III). Because water deficit stress required more time, stress-recovered aspen showed hardly any recovery following 24 h of rewatering in these experiments (Chapter II). Because g_s was used as a measure of the level of water deficit stress, increases in g_s could have influenced the extent of exposure to water deficit stress in some experiments. These changes to the growth chamber were beyond this researcher's control. However, it is suggested that, if it is known that renovations are planned for one of the growth chambers in use, another growth chamber should be used for all experiments, if possible.

A second problem with the equipment was the high-pressure flow meter (HPFM) itself. Use of the HPFM was minimal in this study, because of concerns regarding its reliability in providing accurate and consistent root hydraulic conductivity data (HPFM limitations and sources of error were discussed in Chapter IV). However, E_a measurements were conducted using the HPFM. It was noted that none of the mean E_a values for the different treatment and control groups were significantly different, although the means showed an increase in E_a in water deficit-stressed aspen (Chapter II). This lack of significance was also observed for L_{pr} measurements (Chapters II, III). One other source of error for the HPFM not mentioned in Chapter IV, although it was discussed in Chapter II, was that the HPFM might not be suited for repeated L_{pr} measurements on the same seedling. These repeated measurements are necessary to

obtain L_{pr} values for decreasing temperatures. If tissue rehydration under pressure is occurring, but is not consistent between individual seedlings, then L_{pr} values may increase variably over time, regardless of decreasing temperature. Temperature reduction typically produces lower L_{pr} values based on E_a experiments using pressure chambers (Wan et al. 2001). Furthermore, exposure to high (0.4 MPa) pressure at the end of one L_{pr} measurement, followed by exposure to low (0.01 MPa) pressure at the start of the next L_{pr} measurement could affect flow rate readings at low pressure for all subsequent readings if there is any internal root tissue damage at the higher pressure.

In future experiments, it is suggested that more seedlings per treatment and control group be used if the HPFM is to be used, in an attempt to have a larger sample size to statistical analysis. Additionally, it is also suggested that aspen seedlings be slightly larger, or two weeks to a month older, than they were in the present study to ensure that seedlings are large enough for the adaptor to form a tight seal around the cut section without constricting water flow or damaging xylem tissue. A tight seal is necessary for accurate root water flow measurements with the HPFM.

5.2.3 Experimental Materials

In this study, mercaptoethanol (ME) was used in the mercuric inhibition experiments (Chapters II, III) to reverse mercuric inhibition. A concentration of 50 mM was used, because this was the concentration that had been successfully used in the literature (Wan and Zwiazek 1999). However, this concentration did not reverse mercuric inhibition and in fact, significantly reduced root volume flow density (J_v) (Chapters II, III). Since this study was conducted, results from experiments in this lab

have been performed that show 50 mM is too high a concentration for use and results in reduced root water flow. These experiments show that a value of 5-20 mM ME is best for reversal of mercuric inhibition (Kamaluddin, unpublished results). Experimenting with the optimal concentration of known toxic reagents such as ME can help to minimize any undesired and unexpected effects as a result of concentrations that are not appropriate for use.

5.3 Suggestions for Future Research

Additional research is required in several areas to increase the understanding of the role of roots in regulating water absorption during water stress and stress recovery. Although the present study showed that water deficits affect AQP activity (Chapter II, III), further experimentation is necessary to understand the role of AQPs in regulating root water flow with water deficit stress. Previous studies have shown that some AQPs are deactivated while other AQPs become active during water deficit and salinity (Guerrero et al. 1990, Yamada et al. 1995, Yamada et al. 1997). Some AQPs can be activated by phosphorylation (Daniels et al. 1994, Johansson et al. 1998) while others may have an ion-like gating mechanism (Weaver et al. 1994, Lee et al. 1995). Studies with the objectives of characterizing AQPs located in the root systems of woody plant species, how they are regulated, and whether they are active or not during water deficit stress, would increase the understanding of woody plant root water relations.

Although apoplastic flow rates cannot be quantified by tracer dyes (Hanson et al. 1985, Skinner and Radin 1994, Steudle and Peterson 1998), quantification of apoplastic flow is necessary for understanding how water deficit stress affects root water flow and

AQP activity. The present study showed that apoplastic flow might be important in severely-stressed seedlings and for stress recovery. Changes in apoplastic dye concentration (Chapters II, III) may not indicate actual changes in apoplastic flow because apoplastic dyes tend to underestimate percent apoplastic flow compared to estimates of apoplastic flow conducted with a cell pressure probe. A cell pressure probe has been used to indirectly and reliably measure apoplastic flow (Zhu and Steudle 1991), and seems to be the only reliable method to date. To further understand the role of apoplastic flow, studies that address changes in apoplastic flow with water stress and recovery are needed.

Root anatomy affected root water relations in the present study, as indicated by the comparison of aspen grown in sand and solution culture. Further microscopy of roots subjected to different levels of water deficit stress and recovery would provide useful information about the effect of lateral root formation, tissue thickening and suberization, and the presence of a mature endodermis and exodermis on root water relations and apoplastic flow rates. A previous study (Barrowclough 1998) showed that the extent of mercuric penetration could be observed by exposure of HgCl_2 -treated roots to H_2S , which forms black HgS deposits on root cross-sections. Although this experiment was attempted in control and stressed seedling roots with no conclusive results (data not shown in this study), such a study could provide information about the role of areas of resistance to the movement of HgCl_2 into roots. The exact resistance of the endodermis and exodermis to radial water flow has not been studied because of the difficulty associated with measuring the individual resistances of these tissues (Steudle and Peterson 1998). However, a study that could measure the resistance provided by control

and water deficit-stressed woody plant roots would provide very important information about root resistance to radial flow during water deficit stress.

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